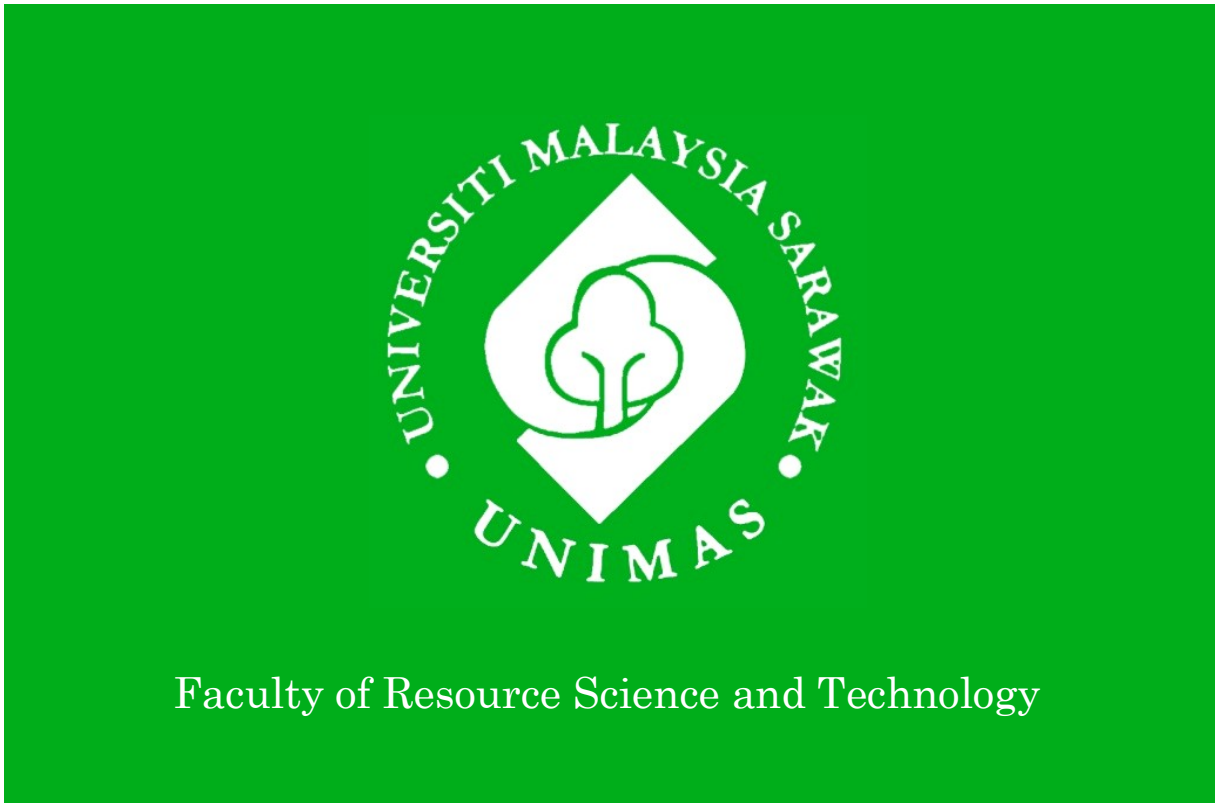

Taxonomy, Pollination Biology and Phylogenetic Analysis of *Schismatoglottis* Calyptrata Complex (Schismatoglottideae: Araceae)

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Taxonomy, Pollination Biology and Phylogenetic Analysis of *Schismatoglottis* Calyptrata Complex (Schismatoglottideae: Araceae)

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DECLARATION

I declare that no portion of this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

_____

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ABSTRACT

Schismatoglottis Calyptrata complex is the most widespread complex in genus *Schismatoglottis* and is extremely variable in vegetative and inflorescence structures. Taxonomy, pollination biology, and phylogeny of ten species of Calyptrata complex were investigated. Eight novel species (*S. adducta* S.Y.Wong & Y.C.Hoe, *S. baangongensis* S.Y.Wong & Y.C.Hoe, *S. caesia* S.Y.Wong & Y.C.Hoe, *S. giamensis* S.Y.Wong & Y.C.Hoe, *S. laxipistillata* S.Y.Wong & Y.C.Hoe, *S. pantiensis* S.Y.Wong & Y.C.Hoe, *S. pseudoniahensis* S.Y.Wong & Y.C.Hoe and *S. roh* S.Y.Wong & Y.C.Hoe) were described; *Schismatoglottis muluensis* M.Hotta is resurrected and *S. calyptrata* (Roxb.) Zoll. & Moritzi is re-circumscribed. The main pollinator for all species of Calyptrata complex is *Colocasiomyia* spp. (Diptera: Drosophilidae); secondary pollinator is *Cycreon* sp. (Hydrophiloideae: Hydrophilidae); opportunist pollinator is *Chaleonus* spp. (Coleoptera: Chrysomelidae) and *Parastasia* spp. (Scarabaeidae: Rutelinae); visitors are *Trigona* sp. (Apinae: Meliponini), Pteromalidae wasps, Chironomidae flies and *Atheta* sp. (Staphylinidae: Aleocharinae, except as secondary pollinator in *S. muluensis*). The floral scent serve to attract the insects and a moderately constricted spathe trapping mechanism serve to retain the visited insects during pistillate anthesis. Gas chromatography-mass spectrometry (GC-MS) analyses revealed all investigated species of Calyptrata complex emitted ester compound class, especially the dominant compound of 3-butenoic acid, 3-methyl-, methyl ester (> 96.35 %) and small relative amount of 2-butenoic acid, 3-methyl-, methyl ester (0.09 – 0.72 %). The floral scent of different inflorescence parts (*S. baangongensis, S. giamensis* and *S. adducta*) showed the appendix emitted the highest total amount (ng/hr) and number of floral compond at period I (0600 – 0800) during pistillate anthesis, followed by spathe, staminate flower zone and

pistillate flower zone. Thermogenesis is biphasic in *S. adducta, S. calyptrata, S. giamensis, S. pseudoniahensis* and *S. roh* Ar1240, with the first peak occurred during pistillate anthesis, the second was during staminate anthesis. *mat*K gene region produced a well resolved tree topology than ITS gene region for phylogeny constructed from 21 species of Calyptrata complex. A reduced phylogeny of eleven accessions representing ten taxa based on *mat*K gene region was used for subsequent locality, insect visitors, floral scent and morphological characters optimization. The tree partially resolved the relationship among species in Calyptrata complex. All species of the Calyptrata complex species are pollinated by *Colocasiomyia* flies and detected to emit 3-butenoic, 3- methyl, methyl ester and 2-butenoic acid, 3-methyl, methyl ester (except floral scent of *S. pesudoniahensis* is not sampled). The species of Calyptrata complex showed distinctive relationships: *Schismatoglottis calyptrata* which was not visited by beetles, has very few interpistillar staminodes (6 – 12); species of Calyptrata complex in Peninsular Malaysia which were visited by *Chaleonus* beetles, bear few interpistillar staminodes (13 – 66); species of Calyptrata complex in Sarawak, which were visited by *Chaleonus* beetles and *Parastasia* beetles (*S. muluensis, S. baangongensis, S. roh* Ar2445 and *S. roh* Ar1240), bear average to many interpistillar staminodes (80 – 469).

Key words: floral odour, *mat*K, molecular phylogeny, pollination, *Schismatoglottis calyptrata* complex, taxonomy, thermogenesis.

Taksonomi, Pendebungaan Biologi dan Analisis Filogenetik* Schismatoglottis *Calyptrata Komplex (Schismatoglottideae: Araceae)

ABSTRAK

Spesies Schismatoglottis *Calyptrata komplex merupakan spesies yang paling luas menyebar dalam genus* Schismatoglottis *dan amat berbeza dari segi struktur vegetatif dan bunga. Taksonomi, kajian pendebungaan dan filogeni dari sepuluh spesies Calyptrata complex dikaji. Lapan spesies baru (*S. adducta *S.Y.Wong & Y.C.Hoe*, S. baangongensis *S.Y.Wong & Y.C.Hoe,* S. caesia *S.Y.Wong & Y.C.Hoe,* S. giamensis *S.Y.Wong & Y.C.Hoe,* S. laxipistillata *S.Y.Wong & Y.C.Hoe,* S. pantiensis *S.Y.Wong & Y.C.Hoe,* S. pseudoniahensis *S.Y.Wong & Y.C.Hoe dan* S. roh *S.Y.Wong & Y.C.Hoe) dihurai;* Schismatoglottis muluensis *M.Hotta dibangkitkan dan* S. calyptrata *(Roxb.) Zoll. & Moritzi dibatasi*. *Agen pendebungaan utama bagi semua spesies Calyptrata komplex ialah* Colocasiomyia spp. *(Diptera: Drosophilidae); agen pendebungaan kedua ialah* Cycreon sp. *(Hydrophiloideae: Hydrophilidae); agen pendebungaan kesempatan ialah* Chaleonus spp. *(Coleoptera: Chrysomelidae) dan* Parastasia spp. *(Scarabaeidae: Rutelinae); pengunjung ialah* Trigona sp. *(Apinae: Meliponini), Pteromalidae tebuan, Chironomidae lalat dan* Atheta sp. *(Staphylinidae: Aleocharinae, kecuali sebagai agen pendebungaan kedua di* S. muluensis*). Bau bunga berfungsi menarik serangga-serangga dan spathe perangkap mekanisme yang sederhana mengekalkan serangga semasa pistillate antesis. Analisis gas chromatography-mass spectrometry (GC-MS) mendedah semua spesies Calyptrata komplex yang dikaji mengeluarkan ester kompaun kelas, terutamanya kompaun utama 3-butenoic acid, 3-methyl-, methyl ester (> 96.35 %) dan relatif amaun 2-butenoic acid, 3-methyl-, methyl ester yang kurang (0.09 – 0.72 %). Bau bunga dari*

*bahagian bunga yang berbeza (*S. baangongensis, S. giamensis *dan* S. adducta*) menunjukkan appendix mengeluarkan paling banyak jumlah amaun (ng/hr) dan bilangan kompaun bunga pada tempoh I (0600 – 0800) semasa pistillate antesis, diikuti dengan spathe, bunga staminate zon dan bunga pistillate zon. Thermogenesis ialah dwifasa pada* S. adducta, S. calyptrata, S. giamensis, S. pseudoniahensis *dan* S. roh Ar1240, *dengan puncak pertama muncul semasa pistillate antesis, kedua semasa staminate antesis. Rantau gen matK menghasilkan satu pokok topologi yang baik diselesai dari rantau gen ITS bagi filogeni yang dibina dari 21 spesies Calyptrata complex. Satu filogeni ringkas dari sebelas aksesi mewakili sepuluh spesies yang berdasarkan rantau gen matK diguna pada ciri-ciri lokasi, serangga, bau bunga dan morfologi optimikasi. Pokok tersebut menyelesaikan sebahagian hubungan di antara spesies dalam Calyptrata komplex. Semua spesies Calyptrata komplex didebungakan oleh* Colocasiomyia *lalat dan dikesan mengeluarkan kompaun 3-butenoic, 3-methyl, methyl ester dan 2-butenoic acid, 3-methyl, methyl ester (kecuali bau bunga* S. pesudoniahensis *tidak disampel). Spesies-spesies dari Calyptrata komplex menunjukkan hubungan yang jelas*: Schismatoglottis calyptrata *tidak dilawat oleh kumbang, mempunyai interpistillar staminodes yang sangat kurang (6 – 12); spesies Calyptrata komplex di Peninsular Malaysia dilawat oleh* Chaleonus *kumbang, mempunyai interpistillar staminodes yang kurang (13 – 66); spesies Calyptrata komplex di Sarawak dilawat oleh* Chaleonus *kumbang dan* Parastasia *kumbang (*S. muluensis, S. baangongensis, S. roh Ar2445 *dan* S. roh Ar1240*), mempunyai sederhana sehingga banyak interpistillar staminodes (80 – 469).*

Kata kunci: *Bau bunga*, *matK, molekul filogeni*, *pendebungaan*, Schismatoglottis calyptrata *komplex*, *taksonomi*, *thermogenesis.*

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LIST OF ABBREVIATION

GC	Gas chromatography
MS	Mass spectrum
GC-MS	Gas chromatography-mass spectrometry
ITS	Internal Transcribed Spacer Region
*mat*K	Megakaryocyte-Associated Tyrosine Kinase
PCR	Polymerase Chain Reactions
oC	Degree Celsius
cm	Centimeter
M	Meter
M	Mole
mm	Milimeter
Um	Micrometer
Min	Minute
mL	Millilitre
mM	Millimolar
uL	Microliter
%	Percent
DNA	Deoxyribonucleic acid
CTAB	Cetyl Trimethyl Ammonium
dNTPs	Deoxynucleotide triphosphates
Taq DNA polymerase	*Thermus aquaticus* DNA polymerase
MgCl2	Magnesium chloride
PVP	Polyvinylpyrrolidone
TBR	Tree-bisection-reconnection
aLRT	Approximate Likelihood-Ratio Test
Bp	Base pair
BPP	Bayesian posterior probability
ML	Maximum likelihood
CI	Consistency index
HI	Homoplasy index
RI	Retention index
RCI	Rescaled consistency index
ILD	Incongruence length difference
asl.	Above sea level
diam.	Diameter
KIs	Kovats Retention Indexes
EI	Electron ionization
PAST	Paleontological Statistics
NMDS	Non-metric multidimensional scaling
SD	Standard deviation
AIC	Akaike information criterion
TVM+G	Transversion Model plus Gamma
TPM1uf+G	Kimura 3-parameter
K81	(Kimura 1981) (TPM1+I+G)
MCMC	Markov chain Monte Carlo

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Araceae (colloquially aroids), is a highly diverse family of monocotyledons herbs comprising an estimated of 140 genera and 6060 species (Boyce & Croat, 2016). Araceae are mostly restricted to the everwet or perhumid tropics Southeast Asia, Malay Achipelago (notably Borneo), tropic Southern Central America and West Africa. Few genera are found in temperate region or at high altitudes in the Andes and Himalaya. Araceae occur as hemiepiphytes, epiphytes, lithophytes, geophytes, rheophytes, helophytes, submerged or free-floating aquatics. Most Araceae species are evergreen, others are seasonally dormant and all are perennial. More than half of the genera have unisexual inflorescences (Aroideae) with the spathe differentiated into spathe limb and lower spathe flower zone; spadix differentiated into pistillate and staminate flower zone. The other genera have bisexual inflorescences (subfamilies Gymnostachydoideae, Orontioideae, Pothoideae, Monsteroideae, Lasioideae, Calloideae) which are simple, naked, with spreading spathe and lacking zone differentiation (spathe and spadix) (Mayo *et al.,* 1997).

The genus *Schismatoglottis* Zoll. & Moritzi is an evergreen tropical aroid that comprises ca 250 species (Boyce & Croat, 2016), with the majority of the species being within Malesia and Borneo, and a very high degree of local endemicity in Borneo. *Schismatoglottis* is the largest genus in tribe Schismatoglottideae Nakai, which comprises other fifteen smaller genera: *Apoballis* Schott (Wong & Boyce, 2010a), *Aridarum* Ridl. (Bogner & Hay, 2000; Wong,

2013), *Bakoa* P.C.Boyce & S.Y.Wong (Boyce & Wong, 2008), *Bucephalandra* Schott (Bogner & Hay, 2000), *Fenestratarum* P.C.Boyce & S.Y.Wong (Boyce & Wong, 2014b), *Galantharum* P.C.Boyce & S.Y.Wong (Boyce & Wong, 2015), *Hestia* S.Y.Wong & P.C.Boyce (Wong & Boyce, 2010a), *Hottarum* Bogner & Nicolson (Low *et al.,* 2011), *Philonotion* Schott (Wong *et al.,* 2010; Cusimano *et al*., 2011), *Ooia* S.Y.Wong & P.C.Boyce (Wong & Boyce, 2010c), *Pichinia* S.Y.Wong & P.C.Boyce (Wong & Boyce, 2010b), *Piptospatha* N.E.Br. (Bogner & Hay, 2000), *Phymatarum* M.Hotta (Bogner & Hay, 2000), *Schottariella* P.C.Boyce & S.Y.Wong (Boyce & Wong, 2009) and *Schottarum* P.C.Boyce & S.Y.Wong (Boyce & Wong, 2008, Low *et al.*, 2013).

Hay & Yuzammi (2000) divided *Schismatoglottis* into six informal species Groups (Asperata, Calyptrata, Corneri, Multiflora, Rupestris and Tecturata). Since then, the Rupestris group was transferred into the genus *Apoballis* (Wong & Boyce, 2010a). For the Calyptrata group, ca 42 species are described so far to belong to this group (Hay & Yuzammi, 2000; Bogner & Boyce, 2009; Wong, 2012; Scherberich & Boyce, 2013). Hay & Yuzammi (2000) determined 36 taxa as conspecific to *S. calyptrata* (Roxb.) Zoll. & Moritzi and therefore were reduced as synonyms. Therefore, with the current delimitation, *S. calyptrata* is the most widespread species in the genus and is extremely variable in terms of leaf shape and inflorescence (Hay & Yuzammi, 2000). In Peninsular Malaysia, a few specimens without the appendix were determined as *S. calyptrata*. In New Guinea, the most robust forms of *S. neoguineensis* (André) N.E.Brown (synonymed as *S. calyptrata*) have ovato-sagittate leaves (up to 40 cm long), the small forms have elliptic leaves (up to 5 cm) and reduced number of interpistillar staminodes that do not exceed the height of pistils (Hay & Yuzammi, 2000). *Schismatoglottis calyptrata* produces synflorescence of up to eight inflorescences that mature sequentially. The

spathe is differentiated into a pronounced spathe constriction coinciding with the base of the staminate flower zone of the spadix. The spadix comprises of appendix, staminate flower zone, interstice and pistillate flower zone. At pistillate anthesis, the spathe limb inflated and opened wide, the spathe constriction has gap to allow access of pollinators. At staminate anthesis, the spathe limb abscises and reveals the staminate flower zone and appendix (Hay & Yuzammi, 2000).

Colocasiomyia de Meijere flies (baechlii group) was considered as the pollinator in *S. calyptrata* (Java and Sumatra) and three *Schismatoglottis* spp. (Java, Sulawesi and Sabah) based on adhered pollen found on *Colocasiomyia* specimens (Sultana *et al.,* 2006; Toda & Lakim, 2011; Fartyal *et al.,* 2013). Few studies concluded *Colocasiomyia* have evolved mutualisms and spend their entire life cycle (egg, larval, pupation, adult feeding and mating) on the inflorescence of *Schismatoglottis* (Sultana *et al.,* 2006; Toda & Lakim, 2011). Recent field pollination studies in tribe Schismatoglottideae have found that *Colocasiomyia* flies (in *Bucephalandra* spp., *S. sarikeense*, *A. nicolsonii*, *P. borneense*) and *Chaloenus* Westwood beetles (in *B. aurantiitheca* S.Y.Wong & P.C.Boyce) as the pollinators and *Chaloenus* spp. beetles (in *S. sarikeense, A. nicolsonii, P. borneense*), *Cycreon* Orchymont beetles (in *S. sarikeense, P. borneense*) and *Altica cyanae* (Weber) beetles (in *S. sarikeense*) as the floral visitors (Low *et al.,* 2013; Wong & Boyce, 2014; Low *et al.,* 2015). Multivariate analyses by Gibernau *et al.* (2010) suggested that the pollinators of *Schismatoglottis* are distributed between flies and beetles.

Floral scent is crucial to indicate the position of the inflorescence under low light condition and attract the pollinator at a long distance (Gottsberger & Silberbauer-Gottsberger, 1991;

Pereira *et al.,* 2014). Several floral scent analyses have been carried out in several genera of Araceae. *Arum maculatum* L. emitted major compounds of *p*-cresol, indole and 2-heptanone (Kite *et al.,* 1998). *p*-cresol was the most effective individual compound, but mixture of *p*-cresol, indole and 2-heptanone attracted more *Psychoda* Latr. midges than any individual or mixture of pair compounds. *Homalomena* sp. majorly emitted 2-butanol (Kumano & Yamaoka, 2006), mixtures including 2-butanol and veratrole attracted the pollinator *Parastasia bimaculata* Guerin scarab beetle (Kumano-Nomura & Yamaoka, 2009). *Philodendron selloum* C.Koch release dominant compound of 4-methoxystyrene, and this compound attracted *Erioscelis emarginata* (Mannerheim) scarab beetle (Gottsberger & Silberbauer-Gottsberger, 1991). Several studies showed floral scent data is significant in species boundary delimitation for angiosperms (Dodson *et al.,* 1969; Williams & Whitten 1999).

Recent phylogeny study for the family of Araceae included tribe Schismatoglottideae (Cusimano *et al.,* 2011). In Schismatoglottideae, Neotropical *Schismatoglottis* was transfered to tribe Philonotieae S.Y.Wong & P.C.Boyce Wong *et al.* (2010). Low *et al.* (2011) transfered misplaced *Hottarum* species to two new genera: *Bakoa* P.C.Boyce & S.Y.Wong and *Ooia* S.Y.Wong & P.C.Boyce. *Schismatoglottis josefii* and *S. sarikeensis* were included in *Schottarum* (Low *et al.,* 2013). In *Schismatoglottis*, only the phylogeny of *Schismatoglottis* Nervosa complex (Ting *et al*., 2012) and one species of *Schismatoglottis* Calyptrata complex were investigated (Barabé, *et al.,* 2004; Wong *et al*., 2010).

1.2 Problem Statements

The species of the Calyptrata complex are morphologically extremely variable especially on its leaf shape and inflorescence structures (Hay & Yuzammi, 2000). Thus, morphological study is required to deal with the taxonomical problem. Previous phylogenetic analyses only involved one species in Calyptrata complex (Barabé *et al.,* 2004; Wong *et al*., 2010). Barabé *et al.* (2004) indicated that Schismatoglottideae is not monophyletic but since then, the neotropical *Schismatoglottis* has been transferred into its own Tribe Philonotion (Wong *et al.,* 2010). The relationship between the species of tropical Calyptrata complex is still unknown. Therefore, molecular study is required to resolve the relationships among the taxa in the Calyptrata complex. The pollination studies of *Schismatoglottis* are still rare. Several studies determined *Colocasiomyia* fly as the pollinator but lacked field observations (Sultana *et al.,* 2006; Toda & Lakim, 2011; Fartyal *et al.,* 2013). Only three field investigations on pollination in tribe Schismatoglottideae were done which recorded *Colocasiomyia* flies as pollinator (Low *et al.,* 2013; Wong & Boyce, 2014; Low *et al.,* 2015) and *Chaloenus* beetle as pollinator (Wong & Boyce, 2014). Floral scent analyses in *Schismatoglottis* have never been studied before. Thus, there is a need to investigate the floral compounds of *Schismatoglottis* and to link it to its pollination strategies. This study was aimed to provide a platform to tie in data on taxonomy, pollination and floral scent onto the phylogeny of the investigated taxa.

1.3 Objectives

The objectives for this study were:

i. To delimitate an informal *Schismatoglottis* Calyptrata complex using morphological characters.

ii. To investigate flowering mechanisms, pollination strategies, and reproductive success of ten investigated species in the Calyptrata complex.

iii. To analyze the similarities and differences in floral scent composition of ten investigated species in the Calyptrata complex.

iv. To resolve the relationship among the 22 taxa in the Calyptrata complex by using ITS and *mat*K regions.

v. To combine the morphological characters, floral scent compounds, and insect visitors onto the phylogenetic tree.

CHAPTER 2

LITERATURE REVIEW

2.1 *Schismatoglottis* Zoll. & Moritzi

The tribe Schismatoglottideae Nakai comprises the genus *Schismatoglottis* Zoll. & Moritzi, and fifteen smaller genera: *Apoballis* Schott (Wong & Boyce, 2010a), *Aridarum* Ridl. (Bogner & Hay, 2000; Wong, 2013), *Bakoa* P.C.Boyce & S.Y.Wong (Boyce & Wong, 2008), *Bucephalandra* Schott (Bogner & Hay, 2000), *Fenestratarum* P.C.Boyce & S.Y.Wong (Boyce & Wong, 2014b), *Galantharum* P.C.Boyce & S.Y.Wong (Boyce & Wong, 2015), *Hestia* S.Y.Wong & P.C.Boyce (Wong & Boyce, 2010a), *Hottarum* Bogner & Nicolson (Low *et al.*, 2011), *Philonotion* Schott (Wong *et al.,* 2010; Cusimano *et al*., 2011), *Ooia* S.Y.Wong & P.C.Boyce (Wong & Boyce, 2010c), *Pichinia* S.Y.Wong & P.C.Boyce (Wong & Boyce, 2010b), *Piptospatha* N.E.Br. (Bogner & Hay, 2000), *Phymatarum* M.Hotta (Bogner & Hay, 2000), *Schottariella* P.C.Boyce & S.Y.Wong (Boyce & Wong, 2009) and *Schottarum* P.C.Boyce & S.Y.Wong (Boyce & Wong, 2008; Low *et al.,* 2013). *Schismatoglottis* is estimated to comprise 250 species, with 119 published species (Boyce & Croat, 2016). Hay & Yuzammi (2000) divided *Schismatoglottis* into six informal species Groups (Asperata, Calyptrata, Corneri, Multiflora, Rupestris and Tecturata). Since then, several new *Schismatoglottis* species (Multiflora Group, Asperata Group and Tecturata Group) were described (Boyce & Wong, 2006; Bogner & Boyce, 2009; Wong, 2010a & b; Wong & Boyce, 2008, 2011; Wong *et al.*, 2012). The species of the Rupestris Group (Hay & Yuzammi, 2000) was transferred into *Apoballis* (Wong & Boyce, 2010a). The neotropical *Schismatoglottis* (*S. americana*) was transferred into *Philonotion* (Wong *et al.,* 2010; Cusimano *et al*., 2011).

Schismatoglottis longifolia Ridl. was transferred into *Hestia* (Wong & Boyce, 2010a). Nervosa complex (Wong, 2010b) and Asperata complex (Boyce & Wong, 2014a) were assigned.

The genus *Schismatoglottis* is widespread from Indochina to Vanuatu, absent from India and Australia, the great majority of the species are Malesian. This genus has a high degree of local endemicity, particularly on Borneo. All species are confined in rainforest areas, from sea level to ca 1,700 m altitude (Hay & Yuzammi, 2000). Most species are terrestrial, except some facultative rheophytic (Boyce & Wong, 2014a) or lithophytic (Wong, 2010b). *Schismatoglottis* is common on steep soil banks of forest, often associated with flowing water. Few species are restricted to particular substrates especially limestone and ultramafic rock (Hay & Yuzammi, 2000; Wong, 2012). *Schismatoglottis* is mostly diminutive except *S. corneri* A.Hay which is a massive arborescent pachycaul. One third of the species are hypogeal stem and with sympodium physiognomically branched. The majority are epigeal with physiognomically unbranched sympodium. The leaves are rather simple with striate venation and much are strikingly variegated, often polymorphic with variegation pattern. The inflorescences are generally small, inconspicuous, the spadix with up to three types of staminodes. The fertile spadix is segregated into staminate and pistillate flower zones that are separated by a sterile flower zone. The spathe often caducous at or soon after anthesis (Hay & Yuzammi, 2000).

2.2 *Schismatoglottis* Calyptrata Group

The *Schismatoglottis* Calyptrata Group is defined by having hapaxanthic shoots, leaf sheath is fully attached to petiole and persistent, and spathe limb caducous at or soon after anthesis. The

group is distributed throughout the Old World. In the last revision of *Schismatoglottis*, 39 species were placed in the Calyptrata Group (Hay & Yuzammi, 2000). Since then, Wong (2012) and Scherberich & Boyce (2013) described a total of three new species of the Calyptrata group (*S. heterodoxa* S.Y.Wong, *S. ranchanensis* S.Y.Wong and *S. scintillans* Scherberich & P.C.Boyce). To date, there are 42 described species for this group in Malesia (Hay & Yuzammi, 2000; Wong, 2012; Scherberich & Boyce 2013).

2.3 *Schismatoglottis calyptrata* (Roxb.) Zoll. & Moritzi

Schismatoglottis calyptrata (Roxb.) Zoll. & Moritzi was described in year 1846 as the type of the genus (Hay & Yuzammi, 2000). The last revision (Hay & Yuzammi, 2000) determined 36 taxa as conspecific to *S. calyptrata* (Roxb.) Zoll. & Moritzi and were reduced as synonyms. This species is widespread from tropical Southwestern China through Indo-China to Vanuatu, found in wet area of Malesia and throughout the whole Borneo. It mostly occurs in lowland and lower montane forests, from sea level to ca 1,700 m altitude. This species is distinguished by its hapaxanthic, hypogeal stems and stoloniferous characters. The plants often grow in colony forms with its morphological variations such as the leaf shape and size is extremely variable and variegated. The leaf is elliptic, with obtuse to cordate base, leaf blade range from 5.5 – 40 cm long. Reduced number of interpistillar staminodes which often do not exceed the height of pistils are usually found in the small plant forms.

2.4 Floral Traits in Araceae

2.4.1 Alimentary Reward

Alimentary rewards of the inflorescence include interpistillar staminodes, staminate synconnective tissues, stigmatic secretions and pollen. Interpistillar staminode can be single clavate (Homalomeneae, *Schismatoglottis*) or various shape-like (*Dieffenbachia* Schott, Spathicarpeae Schott) structure associated with the pistils (Mayo *et al.,* 1997). The interpistillar staminodes of Neotropical *Dieffenbachia* are rich in protein and carbohydrate, and consumed by the scarab pollinator (Young, 1986). This structure is also consumed by beetles (*Parastasia* spp. and *Chaloenus* spp.) that visited inflorescences of *Homalomena* spp. (Kumano & Yamaoka, 2006; Tung *et al.,* 2010; Hoe *et al.,* 2011 & 2016; Hoe, 2012).

Staminate synconnective tissues (Homalomeneae, Philodendreae) and terminal appendix tissues in many taxa (Schismatoglottideae, Areae, Arisaemateae) produce the floral scent to attract pollinators (Vogel & Renner, 1990; Mayo *et al.,* 1997). These structures are frequently chewed by the beetle pollinators and floral visitors (Kato, 1996; Kumano & Yamaoka, 2006; Tung *et al.,* 2010; Hoe *et al.,* 2011 & 2016; Hoe, 2012). Some studies suggest staminate synconnective tissues are not for food reward, but for a better protection of the reproductive organs of the inflorescence (Gottsberger, 1999).

Stigmatic secretions are the extracellular fluid that mostly contains water, sugars, lipids, proteins and other compounds such as amino acids and oil (Konar & Linskens, 1966; Suárez *et al.,* 2012). These secretions probably are the first type of nectar that occurs in many primitive angiosperms (Lloyd & Wells, 1992) and are regarded as a poor substitute for nectar

(Schwerdtfeger *et al.,* 2002). However, Drosophilidae flies have been observed consuming stigmatic secretion and are considered relevant to pollination in *Anthurium* Schott (Croat, 1980; Schwerdtfeger *et al.,* 2002). Large quantities of stigmatic droplets in *A. sanguineum* Engler are consumed by the humming-bird pollinators (Kraemer & Schmitt, 1999).

Several pollination studies in aroids recorded pollen as food reward for the visiting insects (Kumano & Yamaoka, 2006; Franz, 2007; Tung *et al.,* 2010; Hoe *et al.,* 2010; Hoe, 2012). Insect visitors which are found with pollen not effectively adhered onto their bodies are determined as pollen plunderers or visitors (Kumano & Yamaoka, 2006; Hoe, 2012; Hoe *et al.,* 2016). For example, pollen of *Homalomena* that is mixed with sticky resin was not adhere on tiny *Colocasiomyia* de Meijere flies (Tung *et al.,* 2010; Hoe *et al.,* 2010; Hoe, 2012). On the contrary, the powdery pollen of *Alocasia* G.Don (Takenaka *et al.,* 2006) and *Schottarum sarikeense* P.C.Boyce & S.Y.Wong (Low *et al.,* 2013 & 2015) efficiently adhered onto *Colocasiomyia* flies that visiting their inflorescences. Recent study of pollen morphology by using scanning electron microscopy (SEM) and light microscopy (LM) revealed that the pollen of *Schismatoglottis* is psilate (smooth) and that calcium oxalate crystals are irregularly distributed on the pollen surface (Ulrich *et al.,* 2012). Large amount of oxalic acid is deposited as in the form of the crystals of calcium in pollen of most aroids (Mayo *et al.,* 1997).

2.4.2 Reproductive Reward

The insect visitors were recorded to visit the inflorescence of aroids for mating, breeding and ovipositing. Field pollination studies revealed several type of insects mate on the host inflorescences: *Chaloenus* beetles (Kumano & Yamaoka, 2006; Tung *et al.,* 2010; Hoe *et al.,* 2011 & 2016; Hoe, 2012), *Colocasiomyia* flies (Mori & Okada, 2001; Kumano & Yamaoka,

2006; Takenaka *et al.,* 2006; Tung *et al.,* 2010; Hoe *et al.,* 2011 & 2016; Hoe, 2012), scarab beetles (*Parastasia* spp., *Phaeochrous amplus* Arrow, *Cyclocephala* Dejean, *Cyclocephala gregaria* Heyne & Taschenberg and *Cyclocephala amblyopsis* Bates) (Franz, 2006; Kumano & Yamaoka, 2006; Tung *et al.,* 2010; Hoe *et al.,* 2011 & 2016; Hoe, 2012; Young, 1986; García-Robledo *et al.,* 2004) and nitidulid beetles (*Cycreon* sp. and *Colopeterus amputatus* Erichson) (Chouteau *et al.,* 2007; Hoe *et al.,* 2016). Ovipositing was found in *Chaloenus* beetles, *Colocasiomyia* flies, scarab beetles (*Parastasia* spp. and *Cyclanthura carinata*), nitidulid beetle and *Tyloderma* beetles (Gibernau *et al.,* 1999; Franz, 2006; Kumano & Yamaoka, 2006; Takenaka *et al.,* 2006; Tung *et al.,* 2010; Hoe *et al.,* 2011 & 2016; Hoe, 2012). Other studies reported synhospitallic (Okada, 1980 & 1986), stamenicolous (*C. heterodonta* Yafuso & Okada – *Homalomena* spp.) (Yafuso & Okada, 1990), pistillicolous (*C. xanthogaster* Yafuso & Okada– *Homalomena* spp.) (Yafuso & Okada, 1990) and cohabitating (Takano *et al.*, 2011) breeding habits of *Colocasiomyia* flies in aroids. In *Schismatoglottis,* interaction between *Schimatoglottis* and *Colocasiomyia* is regarded as ovipositing mutualism (Chartier *et al.,* 2014).

2.5 Floral Scent

Floral scent predominantly acts as the attractant for the pollinators (Dudareva & Pichersky, 2006; Knudsen *et al.,* 2006). It signals (Pellmyr & Thien, 1986), attracts or guides pollinators to approach, land, seek reward (occasionally mating & reproducing) and cross pollinate the pollen (Raguso, 2001). This signalling cue is potentially more important than visual and tactile cues, and is a subject for co-evolution between plant-pollinator (Kite *et al.,* 1998).

In Araceae, floral scent analyses were mostly investigated in *Arum* L. (Smith & Meeuse, 1966; Kite, 1995; Kite *et al.,* 1998; Stensmyr *et al.,* 2002; Gibernau *et al.,* 2004; Quilichini *et al.,* 2010; Urru *et al.,* 2010; Chartier *et al.,* 2011), *Philodendron* Schott (Gottsberger & Silberbauer-Gottsberger, 1991; Maia *et al.,* 2010; Dötterl *et al.,* 2012; Gottsberger *et al.,* 2013; Pereira *et al.,* 2014), and a few in *Sauromatum* Schott (Borg-Karlson *et al.*, 1994; Hadacek & Weber, 2002), *Anthurium* (Kuanprasert *et al.*, 1998; Schwerdtfeger *et al.,* 2002); *Homalomena* (Kumano & Yamaoka, 2006; Kumano-Nomura & Yamaoka, 2009); *Peltandra* Raf. (Patt *et al.,* 1995); *Amorphophallus* Blume ex Decne. (Kite *et al.,* 1998) and *Alocasia* (Miyake & Yafuso, 2005). Well-developed terminal appendix tissues in some taxa (Areae, Arisaemateae, Colocasieae, Schismatoglottideae, Thomsonieae and Zomicarpeae) or staminate synconnective tissues that overtopped the thecae in several lacking appendix tribes of Aroideae (Philodendreae, Homalomeneae, Anubiadeae, Caladieae, Colocasieae) act as the osmophore function (Vogel & Renner, 1990; Mayo *et al.,* 1997). Specific solitary or compound blends are responsible to attract the specific pollinator. The milk-white coloured adaxial surface of the spathe of *P. adamantinum* Schott might have a visual role at a short distance but without floral scent it is unlikely the insects would ever find the inflorescences from afar (Pereira *et al.,* 2014). Synthetic floral compounds (2-butanol and veratrole) of *Homalomena* sp. attracted the *Parastasia* Westwood pollinator (Kumano-Nomura & Yamaoka, 2009). Other studies in scarab-pollinated plant suggested methoxylated or hydroxylated compounds might be a sex pheromone or aggregation cues to attract both male and female scarab pollinator (Ervik *et al.,* 1999; Ervik & Knudsen, 2003; Knudsen *et al.,* 2006). *Arum maculatum* L. emitted three major compounds (*p*-cresol, indole and 2-heptanone) (Kite *et al.,* 1998). In field, the mixture of *p*-cresol, indole and 2-heptanone attracted more *Psychoda* Latr. pollinator than individual or pair of these three compounds (Kite *et al.,* 1998).

Chartier *et al.,* (2011) found *A. maculatum* attracted more Psychodidae insects than *A. italicum* Mill. In this situation, probably the floral scent of the *A. maculatum* might be a better attractant than *A. italicum* in attracting the Psychodidae (Chartier *et al.,* 2011). The floral scent emitted by the appendix of *A. pictum* might has sex specific properties that attract *Coproica* Rondani females flies and repel the males, or only females being attracted to the oviposition sites (Quilichini *et al.,* 2010). Some aroids deceived the visited insects by producing imitated pheromones or allelochemicals compounds. The oligosulphides scents that mimic decaying meat emitted by *A. maculatum* and *Helicodiceros muscivorus* (L.f.) Engl. dupe the carrion and dung flies to search for food and ovipositor sites in the inflorescence chamber (Kite, 1995; Stensmyr *et al.,* 2002).

2.6 Thermogenesis

Thermogenesis is common in the inflorescence of Araceae (Barabé *et al.,* 2002b). It is exclusively generated in the terminal appendices of spadix and staminate flower zone of species lacking appendices (Mayo *et al.,* 1997). It relates to pollination activities such as promoting spathe opening (Albre *et al.,* 2003), volatilization of floral scents to attract insect visitors (Meeuse & Raskin, 1988; Gottsberger & Silberbauer-Gottsberger, 1991; Mayo *et al.,* 1997; Gibernau & Barabé, 2002; Gibernau *et al.,* 2004; Kumano & Yamaoka, 2006), mating (Seymour *et al.,* 2003), warming (Gibernau & Barabé, 2002; Seymour *et al.,* 2003; Albre *et al.,* 2003), pollen release (Seymour & Schultze-Motel, 1997 & 1999; Gibernau *et al.,* 2000; Albre *et al.,* 2003), resistance to freezing (Camazine & Niklas, 1984) and insect departing (Gibernau *et al.,* 2000; Albre *et al.,* 2003).

Early thermogenesis in Araceae was discovered by Lamarck in 1778 (Proctor *et al.,* 1996). Later, thermogenesis were mostly investigated in *Arum* (Rees *et al.,* 1976 & 1977; Barabé *et al.,* 2002b; Albre *et al.,* 2003; Gibernau *et al.,* 2004; Quilichini *et al.,* 2010; Urru *et al.,* 2010), *Philodendron* (Gibernau & Barabé*,* 2000; Barabé *et al.,* 2002b; Maia *et al.,* 2010; Gottsberger *et al.,* 2013; Pereira *et al.,* 2014), *Sauromatum* (Meeuse, 1975; Meeuse & Raskin, 1988), *Dracunculus vulgaris* Schott (Seymour & Schultze-Motel, 1999), *Alocasia* (Yafuso, 1993), *Homalomena* (Kumano & Yamaoka, 2006) but none in *Schismatoglottis*. Biphasic thermogenic pattern with two major heat production peaks were observed in *Philodendron* (Gibernau *et al.,* 2000; Barabé *et al.,* 2002b; Gibernau & Barabé, 2002; Maia *et al.,* 2010; Pereira *et al.,* 2014) and *Homalomena* sp. (Kumano & Yamaoka, 2006), triphasic (three major heat prodution peaks) in *Dracunculus vulgaris* (Seymour & Schultze-Motel, 1999), and tetraphasic (four major heat production peaks) in *A. maculatum* and *A. italicum* (Albre *et al.,* 2003; Gibernau *et al.,* 2004).

Among the spadix flower zones, the appendix generally produced the highest temperature, followed by staminate flower zone, pistillate flower zone are not documented as thermogenic. Gibernau *et al.* (2004) summarized the heat produced by the appendix of certain *Arum* spp. as high as 15 – 25 °C above the ambient temperature. Albre *et al.* (2003) found that the appendix of *A. italicum* reached to maximum 19.3 °C above the ambient temperature. For spadix lacking an appendix such as *P. melinonii* Brongn. ex Regel and *P. solimoesense*, the staminate flower zone produced the highest temperature (14 – 16.3 °C above the ambient temperature), followed by the sterile staminate flower zone (Barabé *et al.,* 2002b).

2.7 Pollination Investigations in Schismatoglottideae

Pollination investigations in Schismatoglottideae are scarce and only three studies were carried out on native populations (Low *et al.,* 2013 & 2015; Wong & Boyce, 2014). A few studies recorded *Colocasiomyia* flies of the baechlii Group is specialized to *Schismatoglottis* spp. (West Kalimantan, Java, Sulawesi and Sabah) and *S. calyptrata* (Java, Sumatra and Borneo) (Sultana *et al.,* 2006; Toda & Lakim, 2011). Recent field investigations in *Schottarum sarikeense* (Low *et al.,* 2013 & 2015), *Aridarum nicolsonii* Bogner (Low *et al.,* 2015) and *Phymatarum borneense* M.Hotta (Low *et al.,* 2015) marked the *Colocasiomyia* flies as the main pollinator. Other insect visitors were chrysomelid beetles (*Chaloenus* spp. and *Altica cyanae* Weber) and *Cycreon* Orchymont beetles. Wong & Boyce (2014) mentioned seven *Bucephalandra* spp. almost exclusively attracting *Colocasiomyia* flies except *B. aurantiitheca* S.Y.Wong & P.C.Boyce probably pollinated by a single species of Chrysomelidae (Coleoptera) beetle.

2.7.1 Insect Pollinators in Schismatoglottideae

2.7.1.1 Drosophilidae: *Colocasiomyia* de Meijere

Drosophilidae flies is one of the most common insect groups found visiting inflorescence of *Colocasia* Schott*, Culcasia* Palisot de Beauvois*, Furtadoa* M.Hotta*, Homalomena, Nephthytis* Schott*, Schismatoglottis, Spathiphyllum* Schott*, Symplocarpus* Nuttall*,* and *Xanthosoma* Schott (Gibernau, 2003 & 2011). *Colocasiomyia* breeds on living fresh flowers especially in the inflorescence of Araceae. They are different compare to other drosophilid flies that breed on fermenting or decayed organic material of tree sap, leaves and fruits (Okada & Yafuso, 1989; Yafuso & Okada, 1990; Yafuso, 1994; Takenaka *et al.,* 2006; Toda & Lakim, 2011).

Colocasiomyia has been reported specialized to a particular host aroid (Yafuso, 1993; Mori & Okada, 2001; Miyake & Yafuso, 2003; Takenaka *et al.,* 2006; Takano *et al.,* 2011; Toda & Lakim, 2011). The baechlii group of *Colocasiomyia* is specialized to *Schismatoglottis* and *Piptospatha* (Toda & Lakim, 2011). Most *Colocasiomyia* spend their entire life cycles inside the inflorescence and infructescence (Takano *et al.,* 2011). In several aroid species, two *Colocasiomyia* species are documented to visit the same inflorescences but occupying different floral zones. Those species were called "synhospitalic" (Okada, 1980) or in "cohabitation" (Takano *et al.,* 2011). Field pollination investigations observed powdery pollen of *Alocasia* and *Schottarum* (Takenaka *et al.,* 2006; Low *et al.,* 2013 & 2015) adhered on *Colocasiomyia.* However, the sticky resinous pollen of *Homalomena* could not be adhered on *Colocasiomyia* (Tung *et al.,* 2010; Hoe *et al.,* 2011; Hoe, 2012).

2.7.1.2 Scarabaeoideae: Scarabaeidae: Rutelinae: Rutelini: *Parastasia* Westwood

The genus *Parastasia* Westwood is widely distributed from eastern Himalaya to archipelagos (until New Britain island of Papua New Guinea) and from south-western Pacific to southern Japan (Kuijten, 1992). *Parastasia* are phytophagous and its larva lives in dead tree trunks. *Parastasia* have been distributed from Asia to other parts of the world through the transport of cultivated plants, building materials, fire wood, and tree trunks. For example, the Ruteline species have been transported to Hawaii, Fiji, Tonga and Samoa (Ohaus, 1935; Kuijten, 1992).

Scarab beetles form several different families are reported to visit the inflorescences of *Alocasia, Amorphophallus, Anubias* Schott, *Biarum* Schott, *Caladium* Ventenat, *Dieffenbachia, Eminium* (Blume) Schott, *Homalomena, Monstera* Adanson, *Montrichardia* H.Crüger, *Rhaphidophora* Hasskarl, *Sauromatum, Syngonium* Schott, *Typhonium* Schott,

Xanthosoma and *Zantedeschia* K.Sprengel (Gibernau, 2003). Some species are nocturnal (Dynastinae and Rutelinae) (Gottsberger, 1999; Kumano & Yamaoka, 2006), often pollinate flowers with heavy spicy, fruity, or unpleasant floral scents (Gottsberger, 1999; Kumano & Yamaoka, 2006). The Ruteline *Parastasia* beetle pollinated inflorescences with sticky resinous pollen (Kumano & Yamaoka, 2006; Tung *et al.,* 2010; Hoe *et al.,* 2011; Hoe, 2012). Studies reported resinous pollen may ensure better adhered pollen on larger size scarab beetle with poorly haired cuticles (Gibernau & Barabé, 2002).

2.7.1.3 Chrysomeloideae: Chrysomelidae: Alticinae: *Chaloenus* Westwood

The genus *Chaloenus* Westwood was firstly proposed in 1861 (Medvedev, 2004). Medvedev (2004) based on morphological characters proposed 19 species, with the majority of the species recorded in Borneo, and eight from a total of ten Borneon species were endemic. Current revision by Takizawa (2012) described 23 new species from Borneo and Sumatra, and divided this genus into two subgenus: *Chaloenus* Westwood and *Priostomus* Jacoby. The genus *Chaloenus* is the most specious in Borneo, with 36 species among the 42 known species. Many of the species in *Chaloenus* are characterized by sexually dimorphic head shape (Takizawa, 2012).

The subgenus *Chaloenus* spp. seem(s) to be frequent visitors to most mesophytic and rheophytic genera of aroid and found to visit *Homalomena* (Kumano & Yamaoka, 2006; Tung *et al.,* 2010; Hoe *et al.,* 2011; Takizawa, 2012), *Furtadoa sumatrensis* M.Hotta (Mori & Okada, 2001), *Schottarum* (Low *et al.,* 2013 & 2015), *Aridarum nicolsonii* (Low *et al.,* 2015), *P. borneense* M.Hotta (Low *et al.,* 2015), *Schimatoglottis mayoana* Bogner & M.Hotta, *Schimatoglottis wallichii* Hook.f., *Bucephalandra* spp. (Wong & Boyce, 2014), *Ooia*

S.Y.Wong & P.C.Boyce and *Schottariella* P.C.Boyce & S.Y.Wong (Boyce pers. observ.). They were also reported as the pollinator in *B. aurantiitheca* (Wong & Boyce, 2014) and secondary pollinator (Kumano-Nomura & Yamaoka, 2009; Hoe *et al.,* 2011) and pollen plunderers (Tung *et al.,* 2010; Hoe, 2012) in *Homalomena* spp.

2.7.1.4 Hydrophiloideae: Hydrophilidae: Sphaeridiinae: Megasternini: *Cycreon* Orchymont

The Hydrophiloidea is a large group of beetle that is distributed in all parts of the world (Hansen, 1991; Lawrence & Newton, 1995; Archangelsky *et al.,* 2005). It contains ca 475 genera and 6,600 described species (Archangelsky *et al.,* 2005), and the family Hydrophilidae is the most speciose (Lawrence & Newton, 1995; Archangelsky *et al.,* 2005). Hydrophiloidea have a great morphological diversity and tremendous range in size, ranging between 0.5 mm – 5 cm, but the majority between 1.5 – 6 mm. The adults usually feed on plant and decaying organic material. Hansen (1991) classified the genus *Cycreon* Orchymont into the family Hydrophilidae. Recently Short & Fikacek (2013) based on mitochondrial and nuclear genes divided Hydrophilidae into eight subfamilies (Acidocerinae, Chaetarthriinae, Enochrinae, Horelophinae, Horelophopsinae, Hydrophilinae, Rygmodinae and Sphaeridiinae). The adults and larvae of Hydrophiloidea can be found in aquatic (ca 75 %), semiquatic, riparian and terrestrial habitat (Hansen, 1991; Bernhard *et al.,* 2006). Hydrophilidae mostly live in aquatic habitat (Bernhard *et al.,* 2006). However, sub-family Sphaeridiinae (ca. 90 genera) mostly live in terrestrial habitat and rather few species are aquatic/riparian (Jäch, 1998), or substrates with a high water content such as decaying plant material or dung (Bernhard *et al.,* 2006). Recent molecular studies proposed that a Hydropilidae have changed of habitat during their evolution 'from terrestrial to aquatic habitats and back again' (Bernhard *et al.,* 2006).

The genus *Cycreon* Orchymont remains poorly investigated. Orchymont (1919) described the first type species, *C. sculpturatus* in Palembang, Sumatra, Indonesia. Since then, no new species of *Cycreon* is described. Last revision in Hydropilidae genera (Hansen, 1991) was not informative for the genus of *Cycreon* due to no *Cycreon* specimen was accessible, and the revision is based on original designation and monotypy of Orchymont (1919) (Hansen, 1991).

2.7.1.5 Staphylinidae: Aleocharinae: Athetini: *Atheta* Thomson

The family Staphylinidae, or staphylinid rove beetle has ca 500000 species (Newton, 2007). Most staphylinid beetles are predators that predate on insects and other invertebrates. Staphylinid beetles mostly live in forest leaf litter and decaying plant matter (Manley, 1977; Samin & Imani, 2011). Several types of staphylinid beetles live on ocean shores (Samin & Imani, 2011). The Aleocharinae Fleming is the largest subfamily within Staphylinidae, with ca. 1,200 genera and 13,000 species (Thayer, 2005). The tribe Athetini Casey has more than 170 genera (Newton *et al.,* 2000) and the majority of the species are free living, few are associated with ants or termites (Elven, *et al*., 2011). The majority species of the *Atheta* Thomson are generally winged and found in short-lived habitat such as mushrooms, excrements, compost and carrion (Feldmann, 2007). In Southeast Asia, *Atheta* beetles had previously been recorded in Singapore (Cameron, 1920; Sawada, 1987), Borneo: Mount Dulit (Northern Sarawak), Mount Bongo (NW Sarawak), Mouth Kinabalu (Sabah) and Ranau (Sabah) (Sawada, 1980).

Although most staphylinid beetles are predators, however, they may frequently visit the flowers, mainly feeding on pollen and nectar, occasionally causing pollination (Proctor *et al.,* 1996; Gottsberger, 1999). In Araceae, staphylinid beetles were reported in *Lysichiton* Schott,

Symplocarpus, Anthurium, Piptospatha, Chlorospatha Engler, *Amorphophallus, Pseudohydrosme* Engler, *Alocasia, Biarum, Dracunculus* P.Miller, *Eminium* (Blume) Schott, *Typhonium* and *Arum* (Gibernau, 2003 & 2011). Hoe *et al.,* (2011) reported staphylinid as the visitor in *H. debilicrista* Y.C.Hoe. Takano *et al.* (2011) presume that staphylinids target eggs and larvae of *Colocasiomyia* or other insects that breed on the inflorescence of *Alocasia*, *Colocasia* and *Schismatoglottis*.

2.8 Molecular Investigations Using ITS and *matK* Region in Araceae

The *matK* chloroplast gene is approximately 1570 bp in length and codes for a maturase protein. The coding region of *matK* is usually located within an intron of the chloroplast *trnK* gene, except *mat*K encodes tRNALys (UUU) in some ferns (Neuhaus & Link, 1987). *mat*K has a very high evolutionary rate and is usable in phylogenetic reconstructions at high taxonomic levels (order or family) (Müller *et al*., 2006), and sometimes at low taxonomic levels (genus or species) (Hilu *et al*., 2003; Lahaye *et al*., 2008).

The internal transcribed spacers (ITS) sequences vary in length from approximately 500 – 700 bp in angiosperms (Baldwin *et al*., 1995) to 1500 – 3200 bp in some gymnosperms (Liston *et al*., 1996; Maggini *et al*., 2000; Marrocco *et al*., 1996). The ITS are non-coding, located between the 18S and 5.8S coding regions (ITS1) and between the 5.8S and 26S coding regions (ITS2) of nuclear ribosomal DNA (nrDNA) (Wendel *et al.,* 1995). The sequences of ITS regions are more variable than the sequences of the highly conserved coding regions (Booy *et al*., 2000). ITS sequences are suitable for phylogenetic inference at the species, generic or even family levels (Baldwin, 1992; Baldwin *et al*., 1995; Low *et al.,* 2011 & 2013; Wong *et al.,* 2013).

The phylogeny in Araceae has been studied based on chloroplast *trn*L intron (Barabé *et al.,* 2002a; Renner *et al.,* 2004; Cabrera *et al.,* 2008; Low *et al.,* 2013), *trn*L-F intergenic spacer (Barabé *et al.,* 2002a; Grob *et al.,* 2002; Jung *et al.,* 2004; Renner *et al.,* 2004; Rothwell *et al.,* 2004; Tam *et al.,* 2004; Nie *et al.,* 2006; Cabrera *et al.,* 2008; Cusimano *et al.,* 2011; Low *et al.,* 2013), partial *trnK* intron (Cabrera *et al.,* 2008; Cusimano *et al.,* 2011), *rp*l20-*rps*12 spacer (Renner *et al.,* 2004), rpl16 (Gauthier *et al.,* 2008), rbcl (Cabrera *et al.,* 2008; Cusimano *et al.,* 2011), *ndh*F (Nie *et al.,* 2006), external transcribed spacer (ETS) (Gauthier *et al.,* 2008), intergenic spacer trnH-psbA (Low *et al.,* 2013), *matK* (Grob *et al.,* 2002; Gonçalves *et al.,* 2007; Cabrera *et al.,* 2008; Low *et al.,* 2013) and internal transcribed spacer (ITS) (Gauthier *et al.,* 2008; Low *et al.,* 2011; Low *et al.,* 2013; Wong *et al.,* 2013).

Recent molecular study placed tribe Schismatoglottideae within the family of Araceae with supported morphological characters (Cusimano *et al.,* 2011). In Schismatoglottideae, Wong *et al.,* (2010) transferred the Neotropical *Schismatoglottis* to tribe Philonotieae S.Y.Wong & P.C. Boyce by using two plastid markers (*mat*K and *trnL-F*). Low *et al.* (2011) transferred former misplaced *Hottarum* species to two new genera: *Bakoa* P.C.Boyce & S.Y.Wong and *Ooia* S.Y.Wong & P.C.Boyce by using ITS region. Subsequent works by Low *et al.,* (2013) utilized ITS region to transfer *Schismatoglottis josefii* and *S. sarikeensis* to *Schottarum* species. In *Schismatoglottis*, only the phylogeny of *Schismatoglottis* Nervosa complex was studied. The phylogeny resolves the Nervosa complex into two apparent clusters by using *mat*K region (Ting *et al*., 2012).

CHAPTER 3

TAXONOMIC IMPLICATIONS

3.1 Introduction

Last revision by Hay & Yuzammi (2000) proposed Calyptrata Group which is defined by the hapaxanthic shoots, leaf sheath fully attached and persistent, and spathe limb caducous. Within the group, *Schismatoglottis calyptrata* (Roxb.) Zoll. & Moritzi itself is highly variable. *Schismatoglottis calyptrata sensu lato* is distinguished from others in the group by its hapaxanthic, hypogeal stems and stoloniferous, often colony-forming terrestrial habit, the clustered inflorescences on rather short peduncles which elongate and become declinate in fruit, the strongly hourglass-shaped spadix with a poorly defined interstice positioned level with the top of the lower spathe chamber, the scattered (sometimes very few, occasionally absent) interpistillar staminodes about equaling to more usually somewhat exceeding the pistils, the dome-shaped appendix slightly and abruptly wider than the top of the stamiate flower zone. The type of *S. calyptrata* is from Ambon, Maluku, Indonesia (Hay & Yuzammi, 2000).

The ten studied species here are defined within Calyptrata complex: eight species were novel (*S. adducta* S.Y.Wong & Y.C.Hoe, *S. baangongensis* S.Y.Wong & Y.C.Hoe, *S. caesia* S.Y.Wong & Y.C.Hoe, *S. giamensis* S.Y.Wong & Y.C.Hoe, *S. laxipistillata* S.Y.Wong & Y.C.Hoe, *S. pantiensis* S.Y.Wong & Y.C.Hoe, *S. pseudoniahensis* S.Y.Wong & Y.C.Hoe and *S. roh* S.Y.Wong & Y.C.Hoe). *Schismatoglottis muluensis* M.Hotta is resurrected with *Schismatoglottis calyptrata* is re-circumscribed to include only collections from Ambon,

Maluku, Indonesia. However, this chapter does not represent an effective publication of these taxa according to the International Code of Nomenclature for algae, fungi, and plants. The formal publication of all the novel taxa here is currently being undertaken in a separate paper.

3.2 Materials and Methodology

During field sampling, vegetative and reproductive characters of ten species (*S. adducta*, *S. baangongensis*, *S. caesia*, *S. calyptrata, S. giamensis, S. laxipistillata*, *S. muluensis, S. pantiensis*, *S. pseudoniahensis, S. roh* Ar1240 and *S. roh* Ar2445) of Calyptrata complex were measured and recorded (**Table 3.1**) for taxonomical treatment study (Simpson, 2006). The herbarium specimens were vouchered and deposited at Sarawak Forestry Herbarium (SAR). Three living plants of the 22 taxa of Calyptrata complex were collected and cultivated at the Botanical Research Centre, Semenggoh, Kuching, Sarawak.

Table 3.1. List of morphological characters for taxonomical treatment.

Species:	Pistils shape:
Locality:	Pistils colour:
Plant habitat:	Pistils length/width:
Plant height:	Style shape:
Stem type:	Style colour:
Stem size:	Style length/width:
Leaf number:	Stigma shape:
Leaf blade shape:	Stigma colour:
Leaf blade length/width:	Stigma length/width:
Leaf blade apex:	Interpistillar staminode number:
Leaf blade base:	Interpistillar staminode shape:
Leaf blade surface adaxial:	Interpistillar staminode colour:
Leaf blade surface abaxial:	Interpistillar staminode length/width:
Leaf blade midrib:	Interpistillar staminode direction:
Leaf blade primary lateral veins:	Interstice shape:
Leaf blade interprimary veins:	Interstice colour:
Leaf blade secondary veins:	Interstice length/width:
Leaf blade tertiary veins:	Interstice surface:
Leaf blade vein-like pellucid glands:	Staminate flower zone shape:
Petiole shape:	Staminate flower zone colour:
Petiole colour:	Staminate flower zone length/width:

Petiole length/width:	Staminate flower shape:
Petiole surface:	Staminate flower colour:
Petiolar sheath shape:	Staminate flower length/width:
Petiolar sheath colour:	Staminate flower arrangement:
Petiolar sheath length/width:	Pollen type:
Petiolar sheath surface:	Pollen colour:
Inflorescence number:	Appendix shape:
Inflorescence scent:	Appendix colour:
Inflorescence position:	Appendix length/width:
Inflorescence length/width:	Appendix staminode shape:
Peduncle shape:	Appendix staminode colour:
Peduncle colour:	Appendix staminode length/width:
Peduncle length/width:	Appendix staminode arrangement:
Peduncle surface:	Infructescence shape:
Lower spathe shape:	Infructescence position:
Lower spathe colour:	Infructescence length/width:
Lower spathe length/width:	Infructescence surface:
Lower spathe surface:	Fruits shape:
Spathe limb shape:	Fruits colour:
Spathe limb colour:	Fruits length/width:
Spathe limb length/width:	Seed number:
Spathe limb surface:	Seed shape:
Spadix shape:	Seed colour:
Spadix length:	Seed length/width:
Pistillate flower zone shape:	
Pistillate flower zone colour:	
Pistillate flower zone length/width:	

3.3 Results and Discussion

3.3.1 Calyptrata Complex

The Calyptrata complex is defined as stoloniferous herb forming colonies, or clump-forming. Plants are medium to moderately size with hypogeal, hapaxanthic stems. Petiole is terete or D-shaped, smooth with the petiolar sheath fully attached and persistent. Blade is usually ovato-sagittate to ovato-cordate (sometimes oblong-lanceolate with a cordate base), green and sometimes variegated. Primary lateral veins several (12 – 18) per side and irregularly alternating with lesser interprimaries, diverging at 30 – 80 degree, nearly always raised adaxially towards the midrib, marginally impressed, entirely raised abaxially. Secondary

venations arising mostly from the midrib but sometimes from the bases of the primary veins. Tertiary venation is inconspicuous. There are up to as many as eight inflorescences. Each inflorescence emits a strong esteric acid-like scent. The lower spathe is separated from the spathe limb by a constriction coinciding with the upper part of the interstice or the lower part of the staminate flower zone. Spathe limb gaps to reveal an opening ventrally (ca 2 cm across) or with the margins loosely overlapping (5 mm) during pistillate anthesis, closing back and falls fresh at the onset of staminate anthesis. The spadix is sessile, obliquely inserted on the spathe, shorter than spathe, narrowly hourglass-shaped, and divided into four flower zones from base: pistillate flower zone, interstice, staminate flower zone, and appendix. Fruits are as many as 300 – 1,100 per infructescence with ovoid ellipsoid to spherical seeds (4 – 40 per fruit).

Key to the species of *Schismatoglottis* Calyptrata complex

1. Blade leathery ……..…….……3

2. Blade softly coriaceous ……..…….……4

3a. Vein-like pellucid glands not visible; interpistillar staminodes very few (less than 20). Mesophytes, Ambon, Maluku, Indonesia .....*S. calyptrata*

3b. Vein-like pellucid glands visible, interpistillar staminodes many (up to 100). Lithophytes, North Sarawak, Malaysia …..... *S. muluensis*

4a. Leaf blade glaucous abaxially .....*S. caesia*

4b. Leaf blade not glaucous abaxially…….. …..5

5a. Pistillate flower zone laxly arranged .....*S. laxipistillata*

5b. Pistillate flower zone densely arranged 6

6a. Stigma larger than ovary 7

6b. Stigma smaller than ovary 8

7a. Petiole weakly channelled $^{1}/_{6}$ of its length; interstice comprises of staminodes only…….....*S. roh*

7b. Petiole weakly channelled $^{1}/_{3}$ of its length; interstice partially naked with staminodes distally .....*S. giamensis*

8a. Spathe constriction coincides with lower staminate flower zone; interstice not naked; interpistillar staminodes between 30 – 70; appendix between $^{1}/_{7}$ to $^{1}/_{6}$ of spadix length ……9

8b. Spathe constriction coincides with interstice; interstice partially naked; interpistillar staminodes between 80 – 160; appendix ca $^{1}/_{3}$ of spadix length ……..……..10

9a. Vein-like pellucid glands visible; interpistillar staminodes double the height of pistils; interstice with staminodes and pistillodes .....*S. adducta*

9b. Vein-like pellucid gland not visible; interpistillar staminodes similar height to pistils; interstice with staminodes only .....*S. pantiensis*

10a. Staminate flowers bow tie shaped from above, each comprising 2 truncate stamens; staminodes of appendix slender clavate, laxly arranged .....*S. baangongensis*

10b. Staminate flowers densely massed with individual flowers not discernible; staminodes of appendix sub-columnar, sub-globose towards the tip of appendix, densely arranged ……..……..…….*S. pseudoniahensis*

3.4.1 ***Schismatoglottis calyptrata*** **(Roxb.) Zoll. & Moritzi**, Moritzi, Syst. Verz. 83 (1854); Schott, Prodr. Syst. Aroid. 321 (1860); Engl., in D.C. Monogr. Phan. 2:352 (1879); Náves, Noviss. App. 291 (1882); Schumann & Lauterb., Fl. Deutsch. Schutzgeb. Südsee 213 (1901); Usteri, Beitr. Kentnn. Philip. Veg. 130 (1905); Ridl., Materials Fl. Mal. Pen. 31 (1907) & J. Straits Branch Roy. Asiat. Soc. 57:112 (1910) & Fl. Mal. Pen. 5:111 (1925); Engl. & K. Krause, Nova Guinea 8:251 (1910) & in Engl., Pflanzenr. 55:114 (IV.23Da) (1912); Koord., Exkurs.-Fl. Java 1:260 (1911); Merr., Interpr. Herb. Amboin. 129 (1917) & Enum. Philipp. Fl. Pl. 181 (1922); Alderw., Bull. Jard. Bot. Buitenzorg III, 4:212, 343 (1922); Koord., Fl. Tjibodas 6:35 (1922) & Exkurs.-Fl. Java 4:192 (1923) fig. 390; Ochse, Veg. Dutch E. Ind. 60 (1931); Henderson, Mal. Wildfl. Monoc. 231 (1954), fig. 137A; Bakh.f., in Backer, Bekn. fl. Java 17:33 (1957); Bakh.f., & Koster, Blumea 12:67 (1963); Backer & Bakh.f., Fl. Java 3:116 (1968); Chin, Gard Bull. Sing. 35:182 (1982); Peekel, Fl. Bismarck Archip. 70 (1984), fig. 116; Borrell, Checklist Fl. Kairiru Isl. 157 (1989), un-numbered fig.; Hay, Aroids of Papua New Guinea (1990) Pl. 16a, b & Sandakania 7:11 (1996). – *Calla calyptrata* Roxb., Fl. Ind. 3:514 (1832). – *Homalomena calyptrata* (Roxb.) Kunth, Enum. Pl. 3:57 (1841). – [*Colocasia? humilis* Hassk., Flora 25 (2), Beibl. 1:10 (Jul 1842); Tijdschr. Ned. Ind. 4 (2):237 (1842), nom. superfl. pro *Schismatoglottis calyptrata* (based on *Arisarum esculentum* Rumph., Herb. Amboin. 5:111 (1747) t. fig. 1)]. – [*Colocasia? humilis* var. *major* Hassk., Tijdschr. Nat.

Gesch. & Physiol. 9:160 (Aug/Sep 1842); Hassk., Cat. Hort. Bot. Bog. 56 (1844), nom. superfl. pro var. typ.]. – *Zantedeschia calyptrata* (Roxb.) C. Koch, Ind. Sem. Hort. Berol. App. 9 (1854). – [*Schismatoglottis calyptrata* var. *concolor* Hallier f., Bull. Herb. Boiss. 620 (1898); Ridl., Materials Fl. Mal. Pen. 3:31 (1907); Engl., Pflanzenr. 55:115 (IV.23Da) (1912); Ridl., Fl. Mal. Pen. 5:111 (1925), nom. superfl. pro var. typ.]. – Type: *Arisarum esculentum* Rumph., Herb. Amboin. 5:111 (1747) t., fig. 1. (lecto; selected by Hay, 1996). – Epitype: Indonesia, Maluku, Ambon, *Zippelius* s.n. (**Figure 3.1 & 3.2**).

Medium to moderately robust, evergreen, stoloniferous **herb** forming clumps, 50 – 120 cm tall. diverging at 30° – 80° from the midrib, raised adaxially towards the midrib, marginally impressed, entirely raised abaxially; **interprimary veins** adaxially raised adaxially, alternating irregularly with primaries; few (0 – 2) **secondary veins** raised from each primary vein (3 – 4 raised from primary veins near to insertion); **tertiary veins** inconspicuous; **vein-like pellucid glands** not visible. **Inflorescences** up to 3, erect, emit esteric acid-like smell during pistillate anthesis, absent during staminate anthesis; **peduncle** ca 15 cm long x 4 – 6 mm wide, terete, green, erect at anthesis; **spathe** ca 12 cm long; **lower spathe** narrowly ovoid, fresh in a single piece at onset of staminate anthesis; spadix ca 10 cm long, shorter than spathe, ca 3.6 cm long x ca 2.8 cm wide, green, not longitudinally ridged, separated from spathe limb by a constriction coinciding with lower staminate flower zone; **spathe limb** turbinate, ca 8.4 cm long x ca 1.6 cm wide, mucronate for ca 2 mm, pale greenish yellow at pistillate anthesis, pallid prior to staminate anthesis, falls sessile; **pistillate flower zone** sub-cylindric**,** 4 – 4.3 cm long × 8 – 10 mm wide, ca $\frac{2}{5}$ length of spadix, light yellow; **pistils** sub-globose, ca 1.5 mm long x 0.5 – 2 mm wide, somewhat laxly arranged but impressed and laxly arranged distally; **style** barely differentiated; **stigma** sub-globose from above, truncated, smaller than ovary, ca

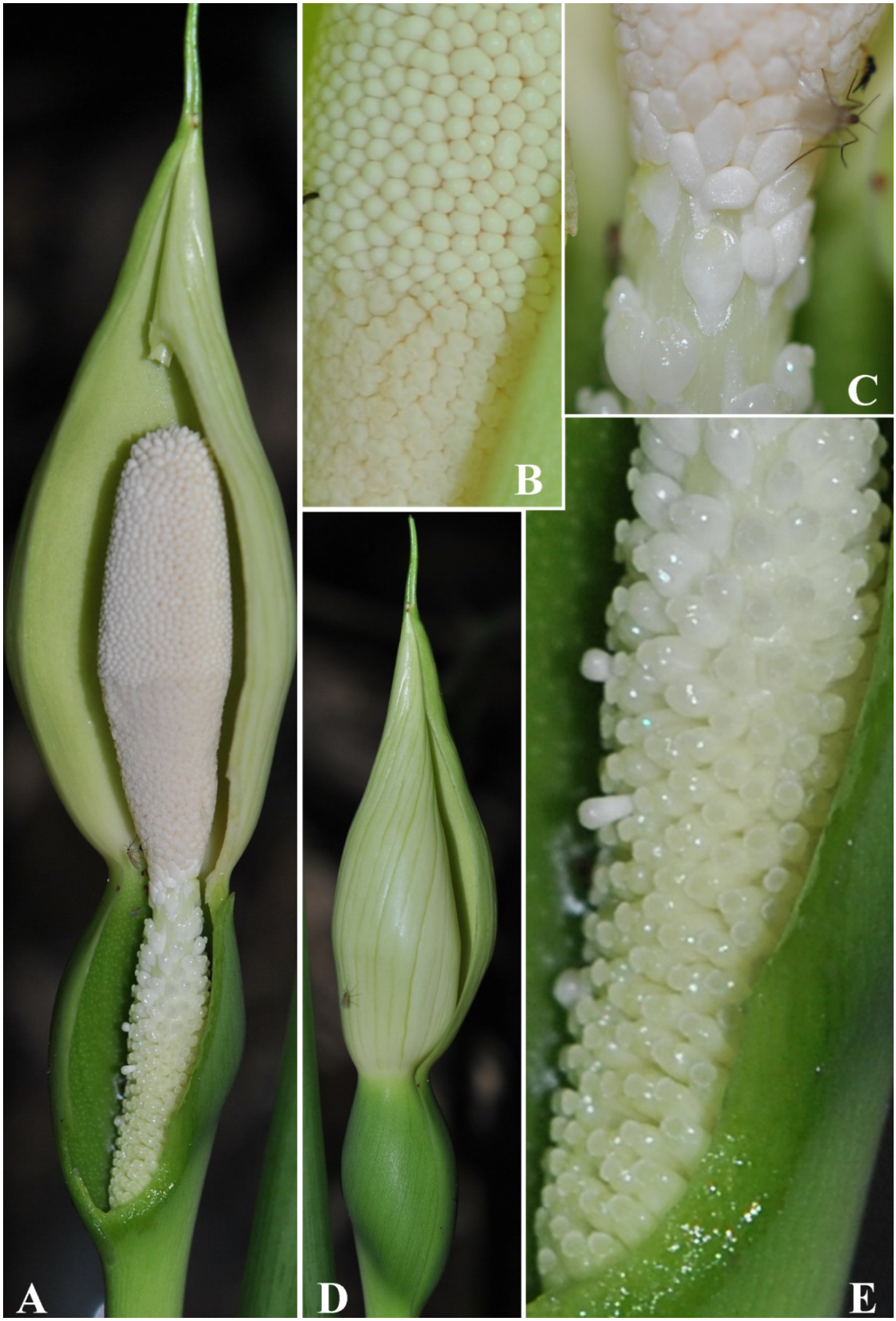

Figure 3.1. *Schismatoglottis calyptrata* (Roxb.) Zoll. & Moritzi. **A**. Inflorescence spadix with spathe was artificially being removed, **B.** Appendix and staminate flower zone; **C**. Interstice; **D.** Inflorescence; **E.** Pistillate flower zone.

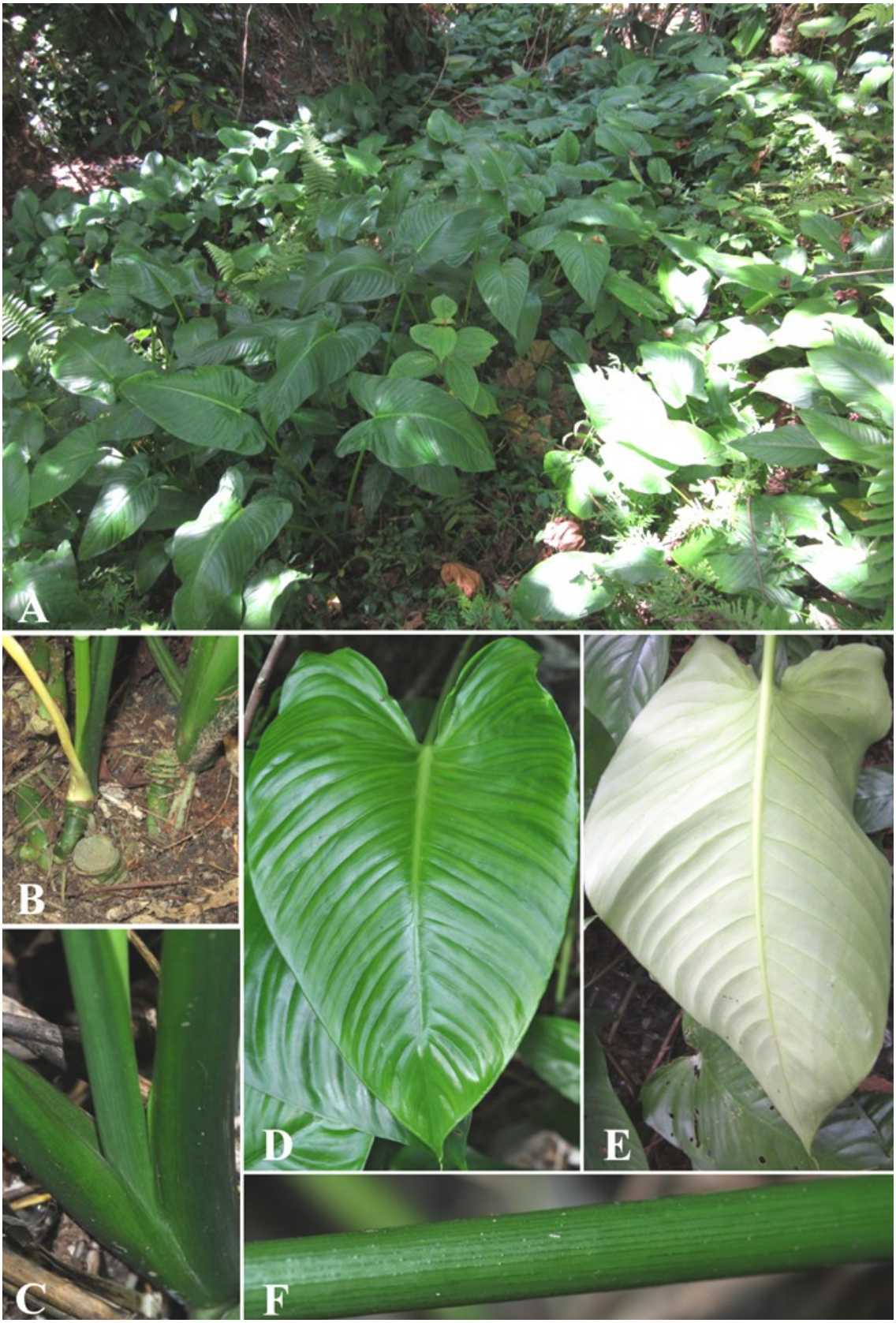

Figure 3.2. *Schismatoglottis calyptrata* (Roxb.) Zoll. & Moritzi. **A.** Plant in habitat; **B**. Hepaxanthic stem; **C**. Petiole bases and the medium green petiolar sheath; **D**. Adaxial leaf; **E**. Abaxial leaf; **F**. Scattered longitudinal medium green striate on petiole.

0.5 mm diam., wet with stigmatic secretion at the onset of pistillate anthesis; **interpistillar staminodes** clavate, stipe slender, very few (6 – 12), ca 3 mm long x 0.6 – 0.8 mm wide, ca double the height of pistils, scattering, white; **interstice** 0.5 – 1 cm long x 4 – 6 mm wide, cylindric, slender than pistillate and staminate flower zone, partially naked, comprised ca two whorls of flattened unequal spheroid staminodes at distal, intergrading into the lower staminate flower zone, pistillodes flattened at proximal, few intergrading into the lower staminate flower zone, light yellow; **staminate flower zone** obconic, narrower at proximal but wider at distal, 2 – 3 cm long × 4 – 12 mm wide, ca $\frac{3}{10}$ length of spadix, yellowish white; **staminate flowers** 1 – 1.8 mm long x 0.5 – 1.5 mm wide, densely massed with individual flowers not discernible except staminate flowers at proximal, bow tie shaped from above, each comprising 2 truncate stamens, sunken in, overtopping by a broad, raised connective; **pollen** powdery, white; **appendix** bullet-shaped, 1.6 – 2.4 cm long x 9 – 13 mm wide, ca $\frac{1}{4}$ length of spadix, base weakly (0.2 – 0.5 mm) wider than apex of staminate flower zone, light yellow; **staminodes** sub-globose, slightly larger and acute towards the tip of appendix, 1 – 2 mm long x 0.5 – 1.5 mm wide, densely arranged. **Infructescences** 1 – 3, 3 – 5 cm long x 1.2 – 1.8 cm wide, declinate; **lower spathe** entirely persistent, reflex when ripe; **fruits** ca 2 mm long x 1 – 3 mm wide, green to yellow; **seeds** ovoid ellipsoid, ca 0.4 mm diam., 8 – 20 per fruit, encased with greenish yellow gel.

Distribution – *Schimatoglottis calyptrata* is endemic to Ambon, Maluku, Indonesia.

Ecology – Lowland tropical forest valley, sandstone interbedded with coral limestone and ambon volcanic rock (andesite, dacite and tuff) (Menzie *et al.,* 1997). 46 m asl.

Notes – Among the studied species, *S. calyptrata* has the fewest number of interpistillar staminodes (6 – 12) and is densely massed with individual flowers which are not discernible except staminate flowers at proximal. *Schismatoglottis calyptrata* and *S. muluensis* share the leathery leaf blade but differs by the laxly arranged pistils in *S. calyptrata*. Pistils of *S. calyptrata* are much longer than *S. muluensis*.

Other specimens seen – Indonesia, Maluku, Ambon, 03.37 S, 128. 19 E, 46 m asl, 8 October 2013, *Y.C.Hoe Ar4270* (SAR); Teluk Ambon, Hative Besar, Jalan Dr. J. Leimena, 03.40 S, 128. 9 E, 26 m asl, 8 October 2013, *Y.C.Hoe Ar4271* (SAR); Teluk Ambon, Hative Besar, Jalan Dr. J. Leimena, 03.40 S, 128.9 E, 26 m asl, 8 October 2013, *Y.C.Hoe Ar4274* (SAR); Teluk Ambon, Rumah Tiga, Jalan Ir. Putuhena, 03.40 S, 128.11 E, 12 m asl, 8 October 2013, *Y.C.Hoe Ar4280* (SAR).

3.4.2 *Schismatoglottis muluensis* M.Hotta, Mem. Coll. Sci. Univ. Kyoto, Ser. B, 32 (1966) 235, fig. 6, A–F. Type. Type: Malaysia, Sarawak, Mardi, western ridge of Gunung Mulu. 17 Mar 1964, *M. Hotta* 14623 (KYO, holo) (**Figure 3.3 & 3.4**).

Medium to moderately robust, evergreen, stoloniferous **herb** forming clumps, 30 – 80 cm tall. **Stems** hypogeal, hapaxanthic, ca 2 cm diam. **Leaves** 3 – 5 together; **petiole** D-shaped, smooth, 34 – 47 cm long, green, weakly channeled ca ½ in its length, longitudinal striates prominent. **Petiolar sheath** 8 – 14 cm long x 5 – 10 mm wide, sheathing for $\frac{1}{4}$ – $\frac{1}{3}$ of petiole length, persistent, membranous, fully attached with a very short ligule, equal at both sides, slightly inrolled or sometimes straight, tapering, scattered greenish dotting striate; **blades** ovato-sagittate to ovato-cordate (sometimes oblong-lanceolate with the base cordate), 25 – 27 cm

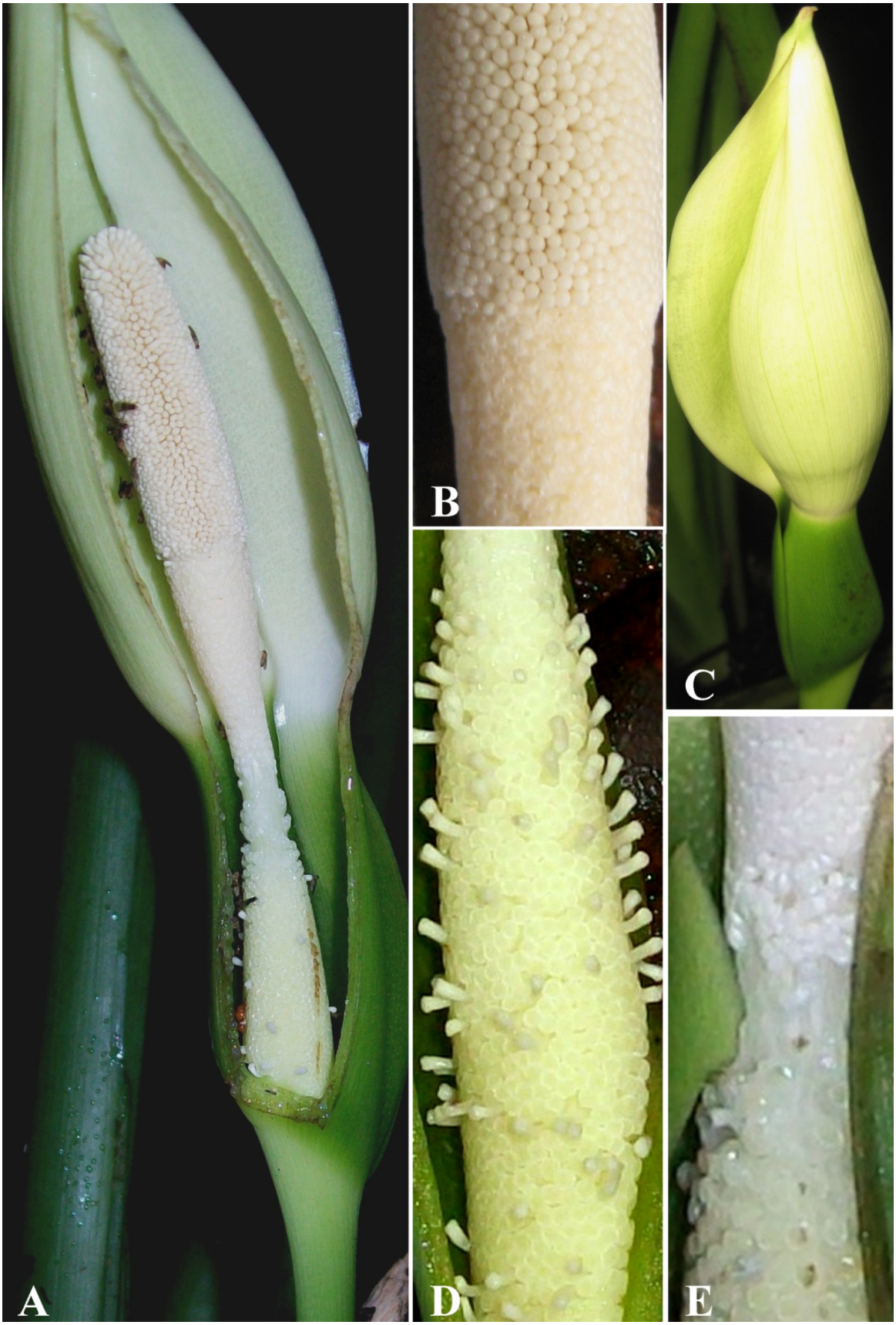

Figure 3.3. *Schismatoglottis muluensis* M. Hotta. A. Inflorescence spadix; B. Appendix and staminate flower zone; C. Inflorescence; D. pistillate flower zone; E. Interstice.

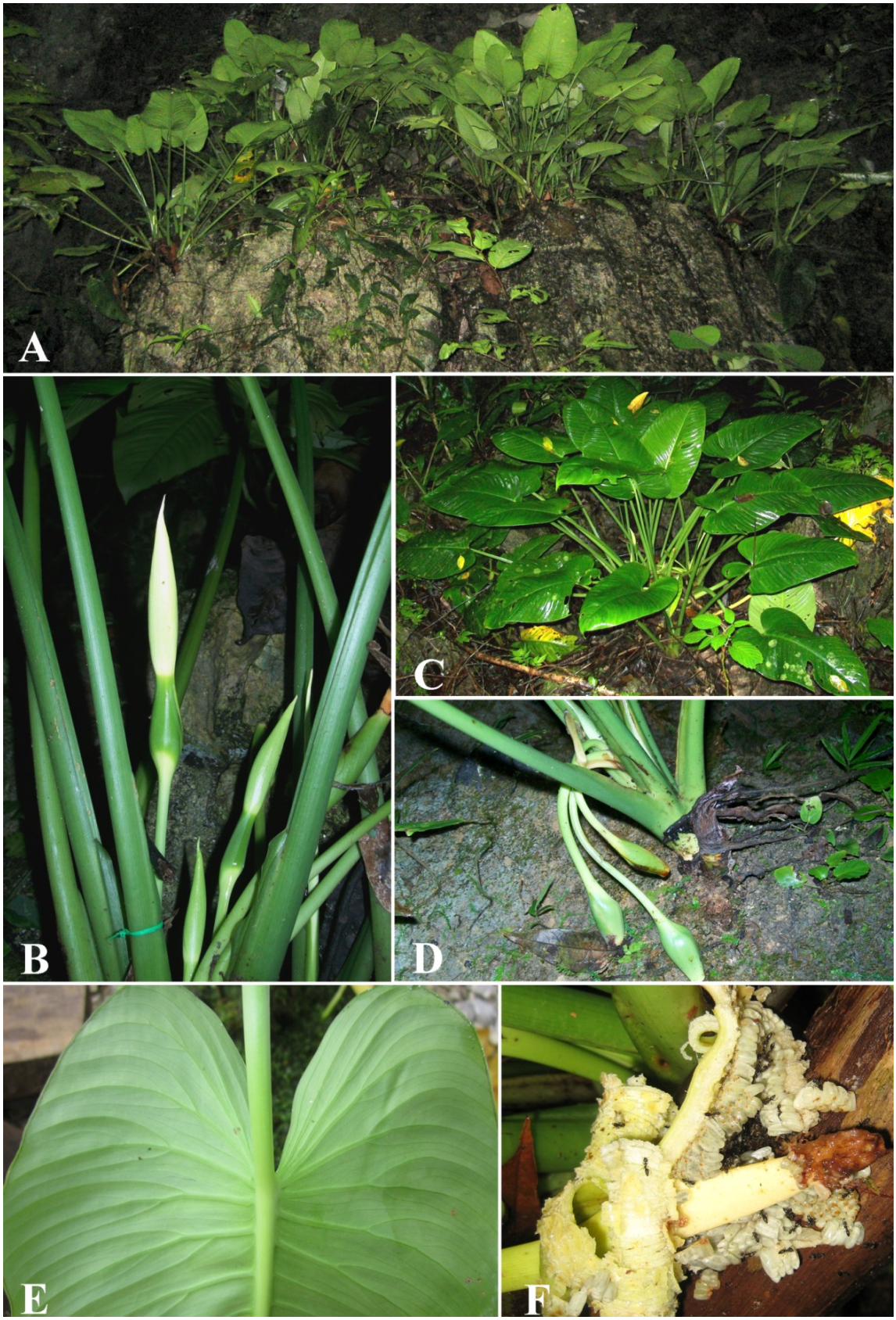

Figure 3.4. *Schismatoglottis muluensis* M. Hotta. A. Lithophyte plants on lime stones; B. synflorescence; C. Plant in habitat; D. Infructescence; E. Abaxial leaf; F. Spathe infructescenes splitting acroscopically and fruits were dispersed by unidentified ants.

long x 13 – 25.5 cm wide, leathery, adaxially glossy green, some variegated with spattered greenish central stripes, abaxially paler, posterior lobes subtriangular for 4.5 – 8 cm, sinus 3.5 – 6 cm across, apex acuminate to acute for 1 – 2 cm, ultimately mucronate for ca 4 mm; **midrib** adaxially flush with blade, raised abaxially, 3.5 – 6 mm at insertion; **primary lateral veins** ca 16, diverging at 30° – 80° from the midrib, raised adaxially towards the midrib, marginally impressed, entirely raised abaxially; **interprimary veins** raised adaxially, alternating irregularly with primaries; 3 – 4 **secondary veins** raised from each primary vein (3 – 4 raised from primary veins near to insertion); **tertiary veins** inconspicuous; **vein-like pellucid glands** clearly visible. **Inflorescences** 1 – 3, erect, emit esteric acid-like smell during pistillate anthesis, absent during staminate anthesis; **peduncle** 10 – 19 cm long x ca 6 mm wide, long, terete, green, erect at anthesis; **spathe** ca 10 cm long; **lower spathe** ovoid-ellipsoid, ca 4 cm long x ca 1.7 cm wide, green, separated from spathe limb by a constriction coinciding with the lower staminate flower zone; **spathe limb** turbinate, ca 6.5 cm long x ca 2.5 cm wide, mucronate for ca 5 mm, pale greenish yellow at pistillate anthesis, slightly pallid prior to staminate anthesis, falls fresh in a single piece at onset of staminate anthesis; **spadix** ca 7 cm long, shorter than spathe, sessile; **pistillate flower zone** cylindric**,** ca 3 cm long × ca 7 mm wide, ca $\frac{2}{5}$ length of spadix, light yellow; **pistils** sub-globose, ca 1 mm long x 0.4 mm wide, densely arranged; **style** barely differentiated; **stigma** sub-globose from above, truncated, larger than ovary, ca 0.5 mm diam., wet with stigmatic secretion at the onset of pistillate anthesis; **interpistillar staminodes** clavate, stipe slender, 80 – 100, ca 0.5 mm in diam., up to double the height of pistils, white; **interstice** cylindric, ca 6 mm long x 5 – 7 mm wide, partially naked, slender than pistillate and staminate flower zone, partially naked, comprised 2 – 5 whorls of flattened spheroid staminodes at distal, intergrading into the lower staminate flower zone, pistillodes flattened at proximal, few pistillodes intergrading into the lower

staminate flower zone, greenish white; **staminate flower zone** sub-cylindrical, narrower at proximal but wider at distal, ca 1.8 cm long × ca 5.5 mm wide, ca $\frac{2}{7}$ length of spadix, yellowish white; **staminate flowers** ca 1 mm long x ca 0.5 mm wide, densely arranged, each comprising 2 truncate stamens, deeply holed, overtopping by a narrow, raised connective; **pollen** powdery, white; **appendix** long cylindric, ca 2 cm long x ca 5.5 cm wide, ca $\frac{2}{7}$ length of spadix, base weakly (0.2 mm) wider than apex of staminate flower zone, yellowish white; **staminodes** sub-globose, rather sub-columnar and weakly protruded at the tip of appendix, ca 1 mm long x 0.5 – 1 mm wide, densely arranged. **Infructescences** 1 – 4, ca 5 cm long x ca 2 cm wide, declinate; **lower spathe** entirely persistent, reflex when ripe; **fruits** ca 2 mm long x ca 1.5 mm wide, green; **seeds** ovoid ellipsoid, ca 0.4 mm diam., 7 – 40 per fruit, with transparent viscous clot, encased with greenish yellow gel.

Distribution – *Schismatoglottis muluensis* is only known from its type locality, trails to Deer Cave & Clear Water Cave, Mulu National Park, Miri Division, Sarawak.

Ecology – On perhumid lowland tropical forests, litophytes on karst limestone, 40 m asl.

Notes – *Schismatoglottis muluensis* is most similar to *S. calyptrata* by its leathery leaf blades but differs from *S. calyptrata* by having stamens which are deeply holed, overtopping by a narrow, raised connective and the other species in the Calyptrata complex by being a lithophyte.

Other specimens seen – Malaysia, Sarawak, Miri, Marudi, Long Lama, Mulu N.P., Trail to Deer Cave, 04. 02 N, 114. 49 E, 60 m asl, 8 August 2006, *P.C.Boyce, S.Y.Wong, Jeland ak*

Kisai & Mael ak Litis Ar1941 (SAR); Marudi, Long Lama, Mulu N.P., Trail from Clearwater Cave, 04.04 N, 114.50 E, 75 m asl, 8 August 2006, *P.C.Boyce, S.Y.Wong, Jeland ak Kisai & Mael ak Litis Ar1964* (SAR); Marudi, Long Lama, Mulu N.P., Trail to Deer Cave, 04. 02 N, 114. 49 E, 60 m asl, 27 September 2007, *P.C.Boyce, S.Y.Wong, I.H.Ooi, Jepom ak Tisai & Mael ak Litis Ar2204* (SAR).

3.4.3 Description of *Schismatoglottis caesia* S.Y.Wong & Y.C.Hoe sp. nov.

Type. Malaysia, Kelantan, Gua Musang, Kuala Koh, Taman Negara Kuala Koh, 04° 52.333'; 102° 26.872', 11 January 2014, Y.C.Hoe Ar4332 (Holotype SAR) (**Figure 3.5 & 3.6**).

Diagnosis

Schismatoglottis caesia S.Y.Wong & Y.C.Hoe is similar to *S. pantiensis* by having a D-shaped and slightly channeled petiole throughout its length, very few interpistillar staminodes (not more than 60) and its height is either equal or slightly exceeding pistils. However, *S. caesia* differs by the glaucous leaf blade abaxially, partially naked interstice with the presence of both pistillodes and staminodes.

Medium to moderately robust, evergreen, stoloniferous **herb** forming clumps, 20 – 60 cm tall. **Stems** hypogeal, hapaxanthic, 0.8 – 1.8 cm diam. **Leaves** 3 – 6 together; **petiole** D-shaped, smooth, 15 – 19 cm long, slightly channeled entirely through its length, green, longitudinal striates prominent distally, darker green; **petiolar sheath**, ca 8 cm long x ca 0.5 wide, sheathing for $\frac{2}{5}$ of petiole length, persistent, membranous, fully attached with a very short ligule, equal at both sides, slightly inrolled or sometimes straight, tapering, scattered greenish dotting striate; **blades** ovato-sagittate to ovato-cordate (sometimes oblong-lanceolate with the

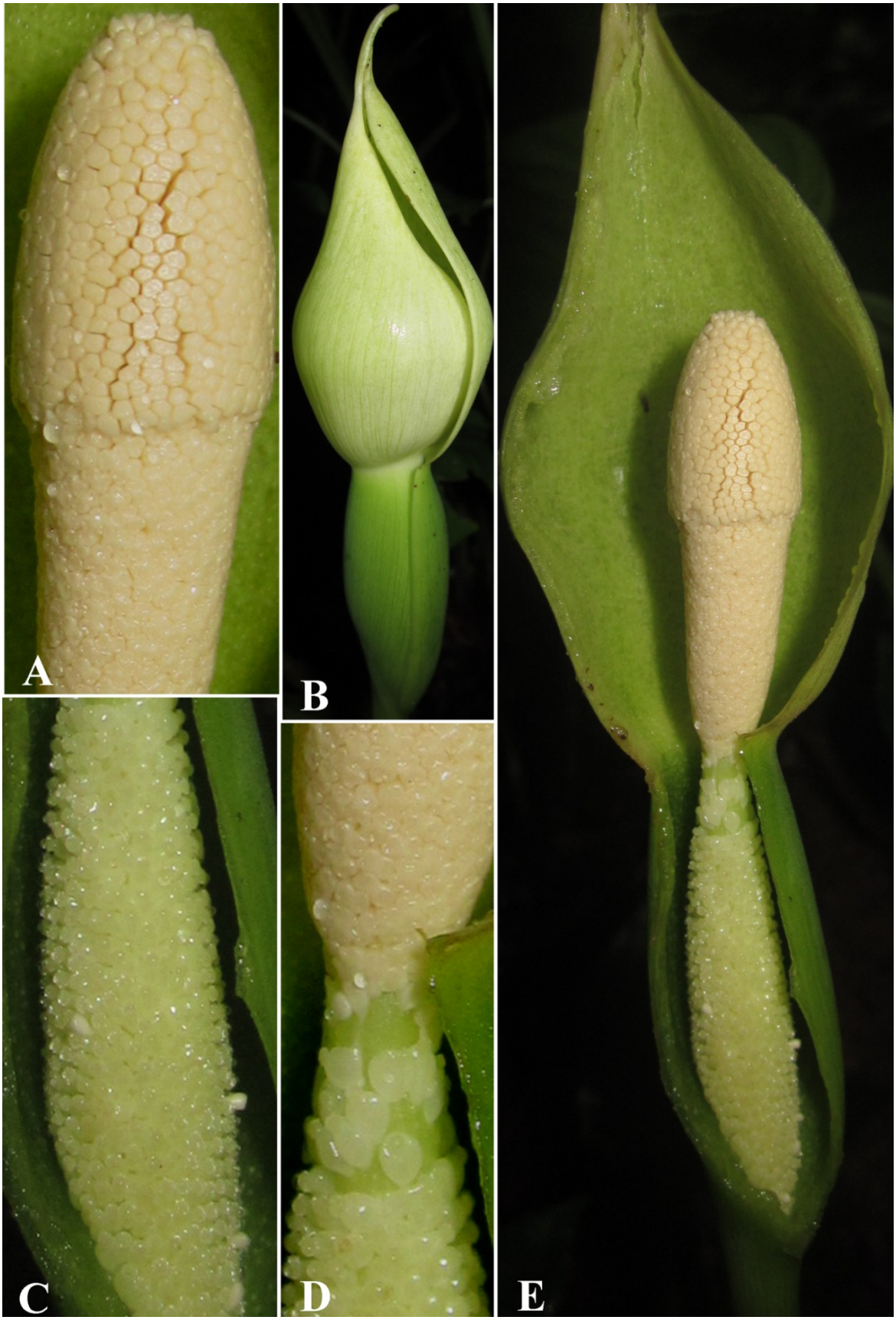

Figure 3.5. *Schismatoglottis caesia* S.Y.Wong & Y.C.Hoe. **A.** Appendix and staminate flower zone. **B.** Inflorescence; **C.** Pistillate flower zone; **D.** Interstice; **E.** Inflorescence spadix.

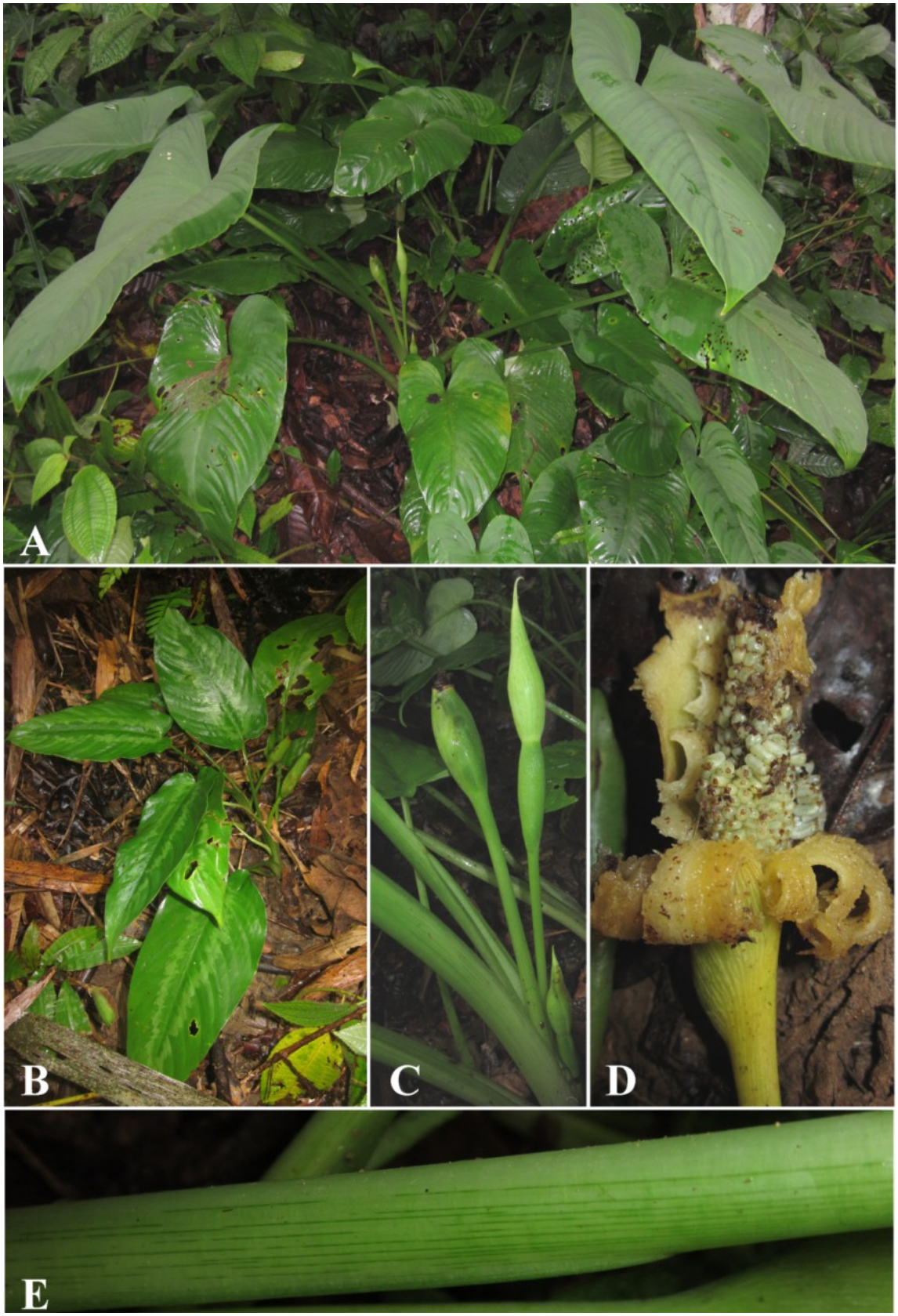

Figure 3.6. *Schismatoglottis caesia* S.Y.Wong & Y.C.Hoe. **A.** Plant in habitat; **B.** Variation of leaf blade; **C.** Synflorescence; **D.** Spathe splitting acroscopically; **E.** Scattered longitudinal medium green striate on petiole.

base cordate), 17.5 – 19 cm long x 7 – 10.5 cm wide, softly coriaceous, adaxially glossy green, some variegated with spattered grey-green central stripes, abaxially glaucous, posterior lobes subtriangular for 3 – 4.5 cm, sinus 4 – 5.5 cm across, apex acute for 2 – 3 cm, ultimately mucronate for ca 2.5 mm; **midrib** adaxially flush with blade, raised abaxially, ca 3.5 mm wide at the insertion; **primary lateral veins** ca 16 per side, diverging at 30° – 80° from the midrib, raised adaxially towards the midrib, marginally impressed, entirely raised abaxially **interprimary veins** raised adaxially, alternating irregularly with primaries; few (0 – 2) **secondary veins** raised from each primary veins (3 – 4 raised from primary veins near to insertion); **tertiary veins** inconspicuous; **vein-like pellucid glands** not visible. **Inflorescences** 1 – 3, erect, emit esteric acid-like smell during pistillate anthesis, absent during staminate anthesis; **peduncle** 10 – 12 cm long x 4 – 8 mm wide, terete, green, erect at anthesis; **spathe** ca 12 cm long; **lower spathe** narrowly ovoid, ca 4.8 cm long x ca 1.8 cm wide, green, longitudinally ridged, separated from spathe limb by a constriction coinciding with lower staminate flower zone; **spathe limb** turbinate, ca 7 cm long x ca 3.8 cm wide, mucronate for ca 4.5 mm, pale greenish yellow at pistillate anthesis, slightly pallid prior to staminate anthesis, falls fresh in a single piece at the onset of staminate anthesis; **spadix** ca 8 cm long, shorter than spathe, sessile; **pistillate flower zone** fusiform, ca 4 cm long × ca 8 mm wide, ca $^{1}/_{2}$ length of spadix, light green; **pistils** sub-cylindric to sub-globose, 1.5 – 2 mm long x 0.6 – 1 mm wide, densely arranged; **style** barely differentiated; **stigma** sub-globose from above, truncated, smaller than ovary, ca 0.3 mm diam., wet with stigmatic secretion at the onset of pistillate anthesis; **interpistillar staminodes** clavate, stipe hardly differentiated, 13 – 38, ca 0.5 mm in diam., slightly exceeding the pistils, scattering, white; **interstice** sub-cylindric, 0.5 – 0.8 cm long x ca 5 mm wide, slender than pistillate and staminate flower zone, partially naked, comprised ca 1 whorls of flattened unequal spherical staminodes, intergrading

into the lower staminate flower zone, pistillodes flattened at proximal; **staminate flower zone** sub-cylindric, narrower at proximal but wider at distal, 1.5 – 2 cm long × 8 – 10 mm wide, ca $\frac{1}{4}$ length of spadix, yellowish white; **staminate flowers** ca 1 mm long x ca 0.5 mm wide, bow tie shaped from above, densely arranged, each comprising 2 truncate stamens, sunken in, overtopping by a broad, raised connective, densely arranged; **pollen** powdery, white; **appendix** bullet-shaped, 1.3 – 1.5 cm long x ca 1 cm wide, ca $\frac{1}{5}$ length of spadix, slightly wider (0.2 – 0.5 mm) than the apex of staminate flower zone, yellowish white; **staminodes** polygonal, sub-globose towards the tip of the appendix, ca 2 mm long x ca 0.5 mm wide, densely arranged. **Infructescences** 1 – 3, ca 4.5 cm long x ca 2 cm wide, declinate; **lower spathe** entirely persistent, reflex when ripe; **fruits** ca 3 mm long x 1 – 2 mm wide, light yellow; **seeds** ovoid ellipsoid, ca 0.4 mm diam., 6 – 35 per fruit, encased with yellow gel.

Distribution – *Schismatoglottis caesia* is only known at the type locality, and trails along Sungai (river) Koh.

Ecology – Perhumid lowland tropical forests, on sandstone, terrestrial along the road margins to steep slope margins of alluvial river stream, 96 m asl.

Etymology – The specific epithet is derived from the latin word, "*caesius'*, bluish grey, referring the glaucous leaf blade abaxially.

3.4.4 Description of *Schismatoglottis laxipistillata* S.Y.Wong and Y.C.Hoe sp. nov.

Type. Malaysia, Kedah, Merbok Division, Bedong, Hutan Lipur Rekreasi Tupah, 05°44.048' 100°26.510', 11 December 2013, Y.C.Hoe Ar4331 (Holotype SAR) (**Figure 3.7 & 3.8**).

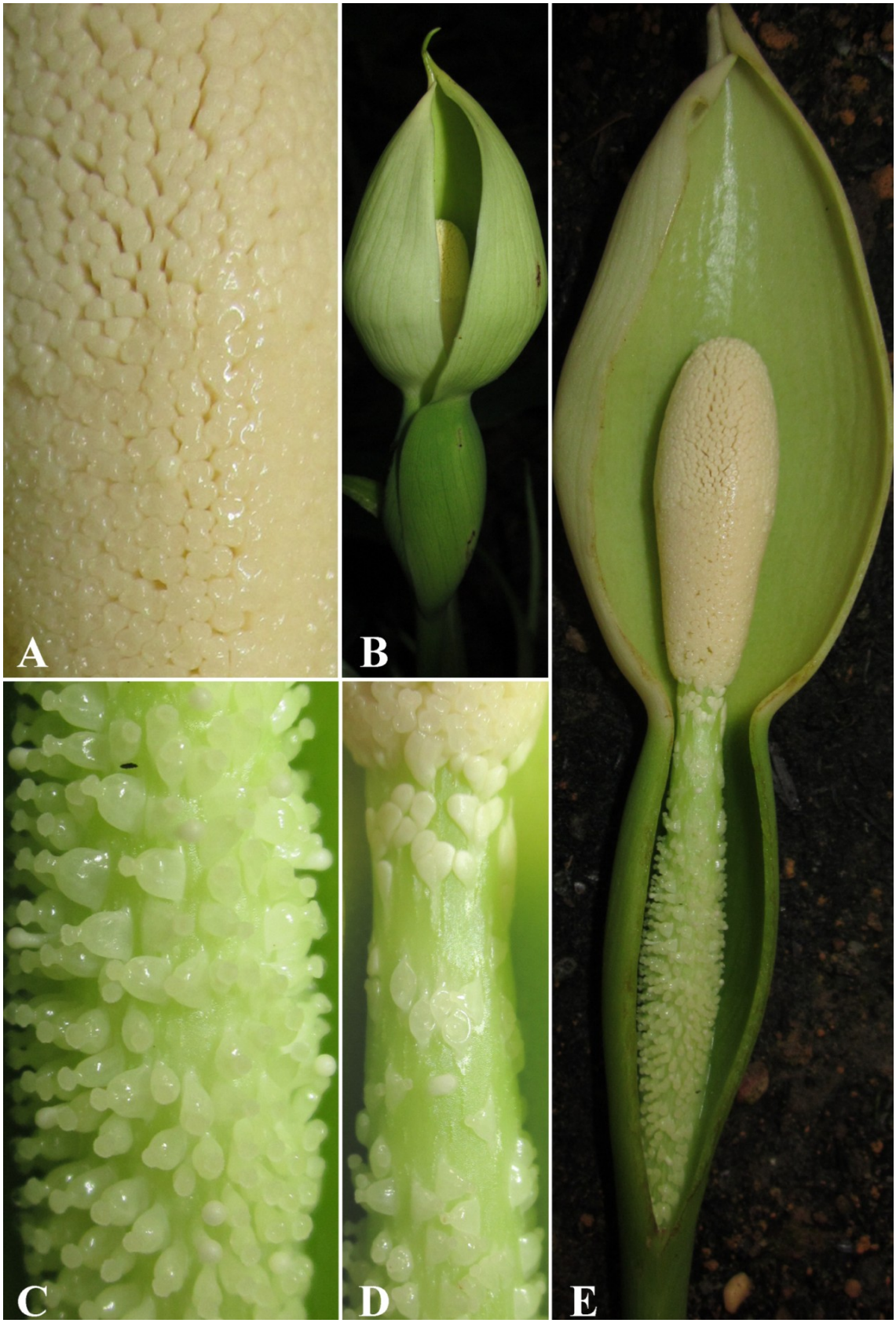

Figure 3.7. *Schismatoglottis laxipistillata* S.Y.Wong & Y.C.Hoe. **A.** Appendix and staminate flower zone. **B.** Inflorescence; **C.** Pistillate flower zone; **D.** Interstice; **E.** Inflorescence spadix.

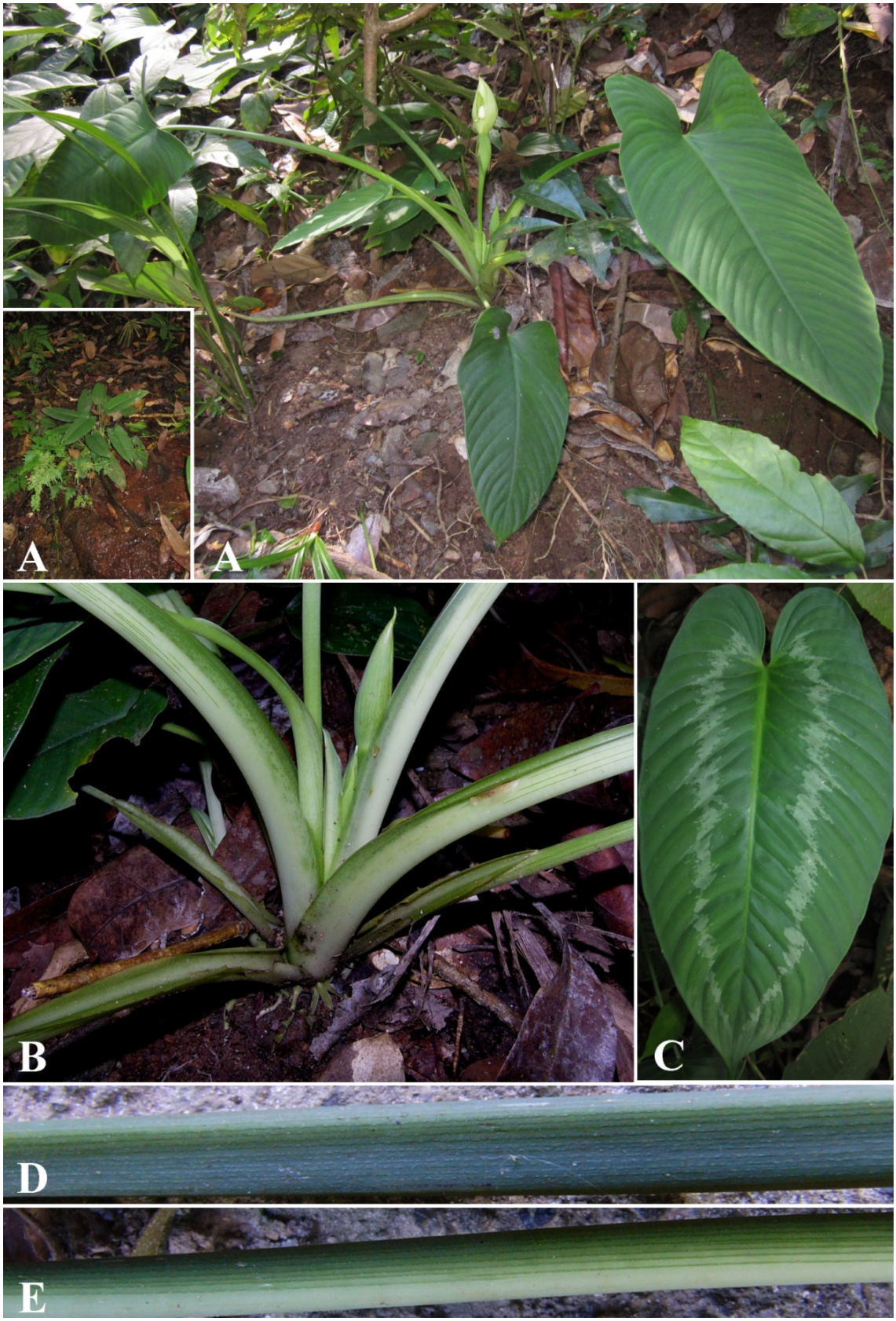

Figure 3.8. *Schismatoglottis laxipistillata* S.Y.Wong & Y.C.Hoe. **A.** Plant in habitat; **B.** Scattered longitudinal medium green striate on petiole; **C.** Leaf variation; **D.** Adaxial petiole slightly groove. **E.** Abaxial petiole greenish white.

Diagnosis

Schismatoglottis laxipistillata S.Y.Wong & Y.C.Hoe are distinguished from the other species in the Calyptrata complex by its laxly arranged pistils.

Medium to moderately robust, evergreen, stoloniferous **herb** forming clumps, 35 – 40 cm tall. **Stems** hypogeal, hapaxanthic, 1 – 2 cm diam. **Leaves** 3 – 8 together; **petiole** D-shaped, smooth, 18 – 20 cm long, green, weakly channeled ca ½ in its length, longitudinal striates prominent distally, darker green; **petiolar sheath** 5 – 8 cm long x 0.5 – 1 cm wide, sheathing for of petiole length, persistent, membranous, fully attached with a very short ligule, equal at both sides, slightly inrolled or sometimes straight, tapering, green with scattered greenish dotting striate; **blades** ovato-sagittate to ovato-cordate (sometimes oblong-lanceolate with the base cordate), ca 29 cm long x ca 16 cm wide, softly coriaceous, adaxially dull green, some variegated with spattered grey-green central stripes, abaxially paler, posterior lobes subtriangular for 3.5 – 5 cm, sinus 3 – 7 cm across, apex acute for ca 2 cm, ultimately mucronate for ca 3 mm; **midrib** adaxially flush with blade, raised abaxially, ca 4 mm at insertion; **primary lateral veins** ca 13 per side, diverging at 30° – 80° from the midrib, raised adaxially towards the midrib, marginally impressed, entirely raised abaxially; **interprimary veins** adaxially raised adaxially, alternating irregularly with primaries; few (0 – 2) **secondary veins** from each primary veins (1 – 2 raised from primary veins near to insertion); **tertiary veins** inconspicuous; **vein-like pellucid glands** not visible. **Inflorescences** up to five, erect, emit esteric acide-like smell during pistillate anthesis, absent during staminate anthesis; **peduncle** 8 – 16 cm long x 3 – 8 mm wide, terete, green, erect at anthesis; **spathe** ca 10.5 cm long; **lower spathe** narrowly ovoid, ca 4.2 cm long x ca 2 cm wide, green, longitudinally ridged, separated from spathe limb by a constriction coinciding with the interstice; **spathe**

limb turbinate, ca 6.5 cm long x ca 3.5 cm wide, mucronate for ca 3 mm, pale greenish yellow at pistillate anthesis, slightly pallid prior to staminate anthesis, falls fresh in a single piece at onset of staminate anthesis; **spadix** ca 8.5 cm long, shorter than spathe, sessile; **pistillate flower zone** fusiform, ca 4 cm long × ca 7 mm wide, ca $\frac{1}{2}$ length of spadix, light green; **pistils** sub-cylindric to sub-globose, ca 1.5 mm long x 0.5 – 1 mm wide, laxly arranged; **style** barely differentiated; **stigma** globose from above, truncated, smaller than ovary, ca 0.3 mm diam., wet with stigmatic secretion at the onset of pistillate anthesis; **interpistillar staminodes** clavate, stipe slender, 17 – 65, ca 0.5 mm in diam., only slightly taller than pistils, scattering, white; **interstice** sub-cylindric, 0.6 – 1 cm long x ca 5 mm wide, slender than pistillate and staminate flower zone, partially naked, few flattened irregular spheroid staminodes closely packed at proximal, partially intergrading into the lower staminate flower zone, few pistillodes flattened at proximal; **staminate flower zone** obconic, narrower at proximal but wider at distal, ca 1.8 cm long × ca 10 mm wide, ca $\frac{1}{4}$ length of spadix, yellowish white; **staminate flowers** ca 1 mm long x ca 0.5 mm wide, bow tie shaped from above, densely arranged, each comprising 2 truncate stamens, connected by a broad connective, densely arranged, yellowish white; **pollen** powdery, white; **appendix** bullet-shaped, ca 1.6 cm long x ca 1 cm wide, ca $\frac{1}{5}$ length of spadix, equal or weakly (0.2 mm) wider than apex of staminate flower zone, yellowish white; **staminodes** sub-globose to polygonal, ca 2 mm long x 0.4 – 1 mm wide, densely arranged. **Infructescences** 1 – 5, ca 5 cm long x ca 2 cm wide, declinate; **lower spathe** entirely persistent, reflex when ripe; **fruits** ca 3 mm long x 1 – 2 mm wide, light yellow; **seeds** ovoid ellipsoid, ca 0.4 mm diam., 4 – 8 per fruit, with greenish yellow gel.

Distribution – *Schismatoglottis laxipistillata* is only known from its locality at Hutan Lipur Rekreasi Tupah, Merbok Division, Kedah.

Ecology – On lowland tropical forest, occurring beside the margin of the waterfall stream and restricted on a 150 m^2 steep granite slopes that are slightly inundated with sandstone mud. 91 m asl.

Etymology – The specific epithet is referring to the laxly arranged pistils.

3.4.5 Description of *Schismatoglottis roh* S.Y.Wong & Y.C.Hoe sp. nov.

Type. Malaysia, Sarawak, Kuching Division, Bau, Krokong, Fairy cave, 01° 22' 51.9"; 110° 07' 09.3", 9 May 2009, P.C.Boyce & S.Y.Wong Ar2445 (Holotype SAR) (**Figure 3.9 & 3.10**).

Diagnosis

Schismatoglottis roh S.Y.Wong & Y.C.Hoe is most similar to *S. giamensis* by having stigma larger than ovary and staminodes at interstice are similar to interpistillar staminodes. However, *S. roh* differs from *S. giamensis* by having petiole weakly channeled up to $^1/_6$, the petiolar sheath up to ¼ of its length and the interstice is not partially naked.

Medium to moderately robust, evergreen, stoloniferous **herb** forming clumps, ca 70 – 85 cm tall. **Stems** hypogeal, hapaxanthic, 1 – 2 cm diam. **Leaves** 3 – 4 together; **petiole** terete, smooth, 32 – 70 cm long, green, weakly channeled ca $^1/_6$ in its length, longitudinal striates prominent distally, darker green; **petiolar sheath** 9 – 15 cm long x 0.5 – 1 cm wide, sheathing for $^1/_6$ to $^1/_4$ of petiole length, persistent, membranous, fully attached with a very short ligule, equal at both sides, slightly inrolled or sometimes straight, tapering, longitudinal striates visible; **blades** ovato-sagittate to ovato-cordate (sometimes oblong-lanceolate with the base cordate), 19 – 35 cm long x 9.5 – 19.5 cm wide, softly coriaceous, adaxially glossy green, few

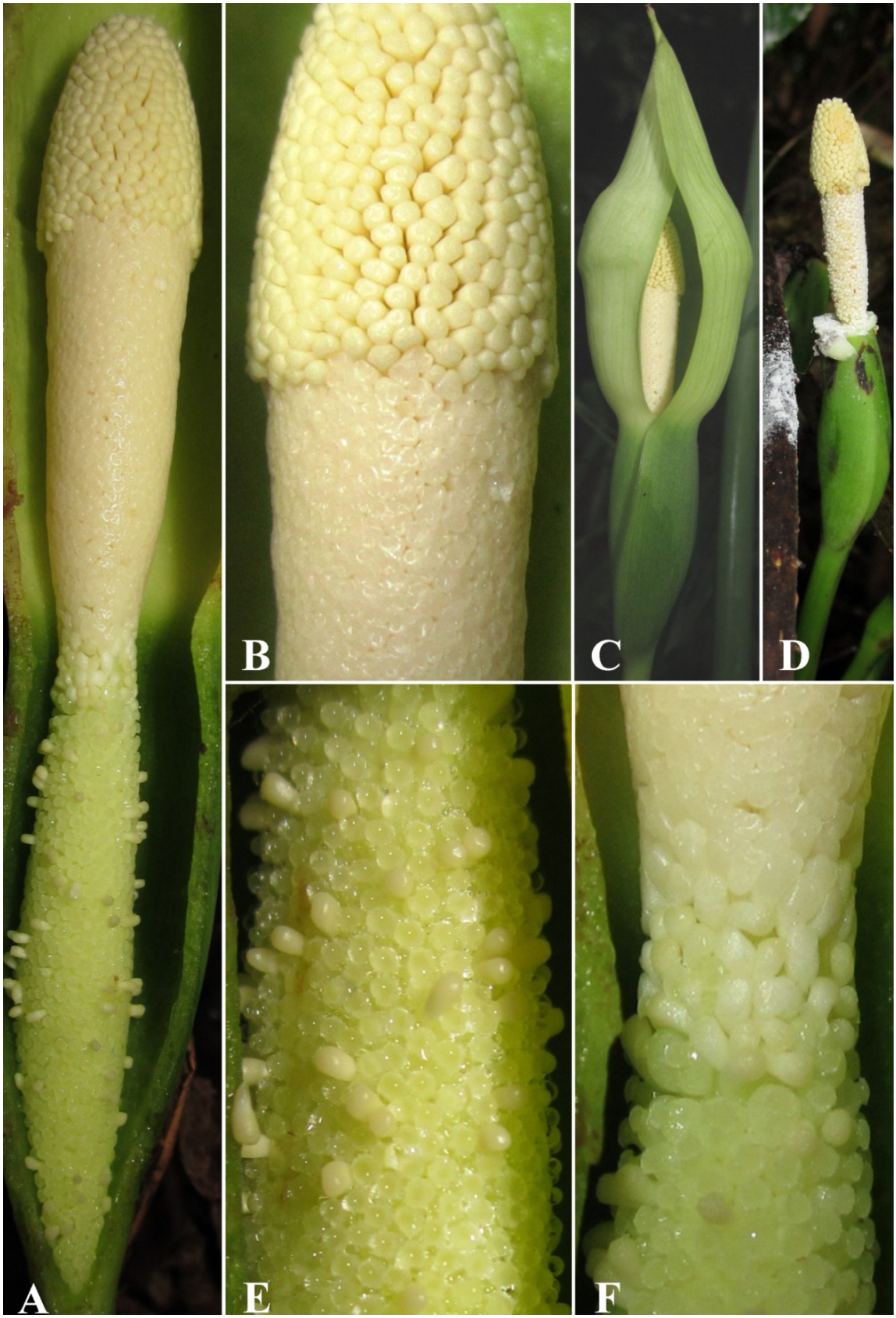

Figure 3.9. *Schismatoglottis roh* Ar2445 S.Y.Wong & Y.C.Hoe. **A.** Inflorescence spadix; **B.** Appendix and staminate flower zone; **C.** Inflorescence; **D.** Pollen release; **E.** Pistillate flower zone; **F.** Interstice.

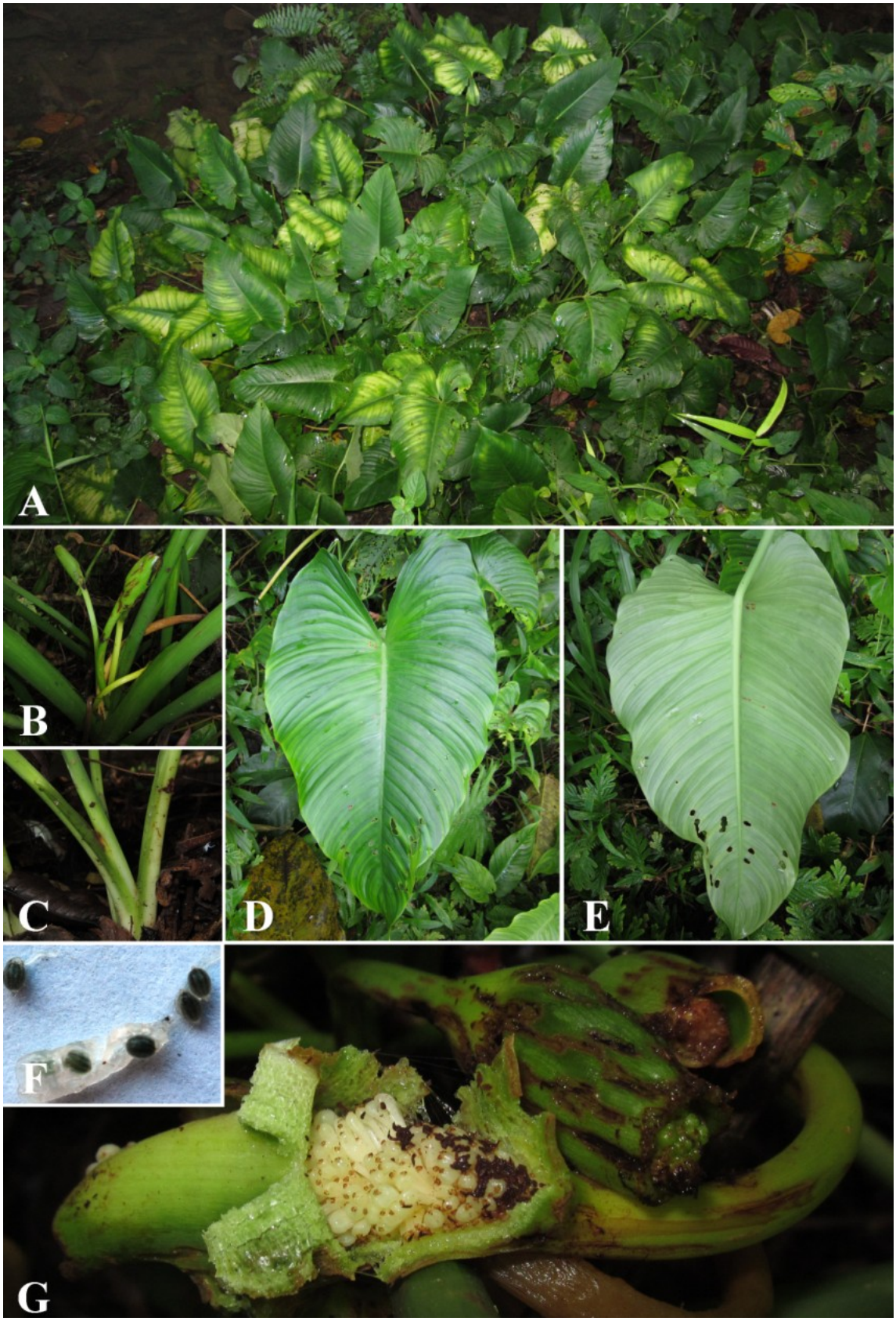

Figure 3.10. *Schismatoglottis roh* Ar2445 S.Y.Wong & Y.C.Hoe. **A.** Plant in habitat; **B.** Synflorescence; **C.** Pale green petiole; **D.** Adaxial leaf; **E.** Abaxial leaf; **F.** Germinating seeds in a fruit capsule; **G.** Fruiting spathe dehisces.

variegated with yellowish bands, abaxially paler, posterior lobes subtriangular for 8 – 11.5 cm, sinus 7 – 10 cm across, apex diverging at 30° – 80° from midrib, raised adaxially towards the midrib, marginally impressed, entirely raised abaxially; **interprimary veins** raised adaxially, alternating irregularly with primaries; few (0 – 2) **secondary veins** raised from each primary vein (3 – 4 secondary veins raised from primary veins near to insertion); **tertiary veins** inconspicuous; **vein-like pellucid glands** visible. **Inflorescences** up to three, erect, emit esteric acid-like smell during pistillate anthesis, absent during staminate anthesis; **peduncle** 10 – 12 cm long x 3 – 5 mm wide, terete, acute for 1 – 2 cm, ultimately mucronate for 5 – 10 mm; **midrib** adaxially flush with blade, raised abaxially, 4 – 5 mm wide at the insertion; **primary lateral veins** 16 – 18 per side, green, erect at anthesis; **spathe** 9 – 12.5 cm long; **lower spathe** narrowy ovoid, 3.8 – 4 cm long x 1.8 – 2.2 cm wide, green, longitudinally ridged, separated from spathe limb by a constriction coinciding with lower staminate flower zone; **spathe limb** turbinate, 6 – 8.5 cm long x 2.5 – 3 cm wide, mucronate for ca 5 mm, pale yellowish green at pistillate anthesis, pallid prior to staminate anthesis, falls fresh in a single piece at the onset of staminate anthesis; **spadix** 7.5 – 9.5 cm long, shorter than spathe, sessile; **pistillate flower zone** weakly fusiform, 3 – 4 cm long × 7 – 11 mm wide, ca $\frac{2}{5}$ length of spadix, apex slightly tapering, light green; **pistils** sub-globose, ca 1 mm long x 0.4 mm wide, densely arranged; **style** barely differentiated; **stigma** globose from above, truncated, weakly wider than ovary, ca 0.5 mm diam., wet with stigmatic secretion at the onset of pistillate anthesis; **interpistillar staminodes** clavate, stipe stout, 100 – 469, 0.5 – 1 mm in diam., ca double the height of pistils, scattering, white; **interstice** sub-cylindric, ca 5 long x ca 4 mm wide, slender than pistillate and staminate flower zone, not naked, comprised 2 – 4 whorls of clavate staminodes, staminodes and pistillodes not impressed, ca 1 mm long x 0.5 – 0.8 mm wide, densely packed, white; **staminate flower zone** sub-cylindric, narrower at proximal but

wider at distal, 2.2 – 3 cm long × 7 – 10 mm wide, $\frac{1}{4} - \frac{1}{3}$ length of spadix, yellowish white; **staminate flowers** ca 1 mm long x ca 0.5 mm wide, densely arranged, bow tie shaped from above, each comprising 2 truncate stamens, sunken in, separated by a narrow connective, densely arranged; **pollen** powdery, white; **appendix** bullet-shaped, 1.5 – 2 cm long x 0.8 – 1.1 cm wide, ca $\frac{1}{5}$ length of spadix, base (ca 0.5 mm) wider than apex of staminate flower zone, yellowish white; **staminodes** sub-globose, ca 2 mm long x 0.5 – 1.5 mm wide, densely arranged. **Infructescences** 1 – 3, 4 – 5.3 cm long x 1.5 – 2 cm wide, declinate; **lower spathe** entirely persistent reflex when ripe; **fruits** 2 – 4 mm long x 1 – 2.5 mm wide, green to yellow; **seeds** ovoid ellipsoid, ca 0.4 mm diam., 4 – 18 per fruit, encased with greenish yellow gel.

Distribution – *Schismatoglottis roh* is known from limestone areas at Bau, Kuching Division, Sarawak.

Ecology – Perhumid lowland tropical forest, on karst limestones. 30 – 45 m asl.

Etymology – The specific epithet is derived from the Malay word, *'roh'* which means soul, referring to the spirit of the fairies at Fairy Caves (Gua Peri-Peri).

Notes – The collections from Gua Angin (Wind Cave) differs by having more interpistillar staminodes (up to 470) (**Figure 3.11 & 3.12**).

Figure 3.11. *Schismatoglottis roh* Ar1240 S.Y.Wong & Y.C.Hoe. A. Inflorescence spadix; B. Inflorescence; C. Interstice and pistillate flower zone; D. Appendix and staminate flower zone.

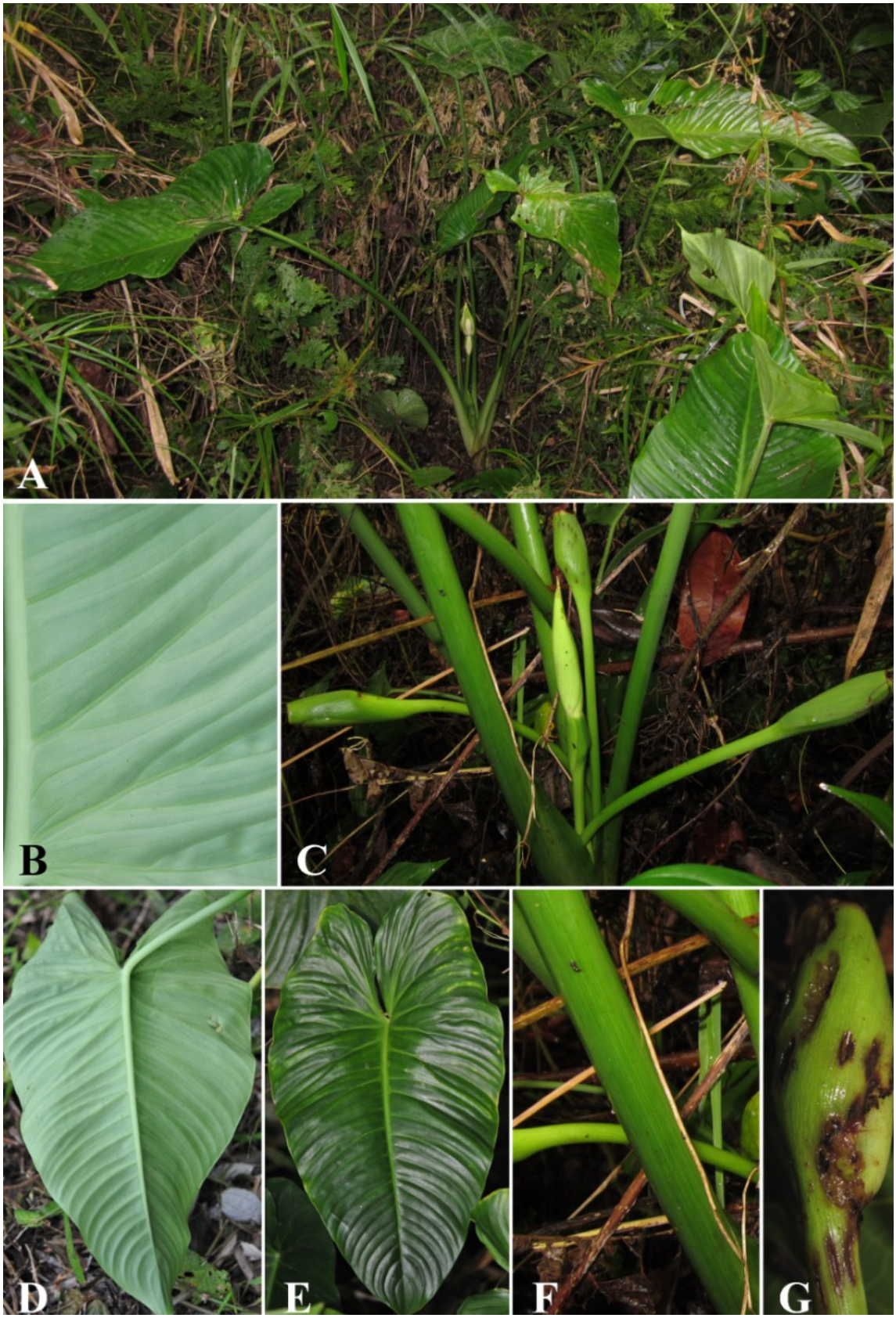

Figure 3.12. *Schismatoglottis roh* Ar1240 S.Y.Wong & Y.C.Hoe. A. Plant in habitat; B. Venation on the abaxial leaf; C. Synflorescence; D. Abaxial blade; E. Adaxial blade; F. Scattered longitudinal medium green striate on petiole; G. Infructescence.

Other specimens seen – Malaysia, Sarawak, Kuching Division, Bau, Wind cave, 01° 24’ 54.8”; 110° 08’ 08.2”, 21 June 2005, *P.C.Boyce & Jeland ak Kisai Ar1240* (SAR).

3.4.6 Description of *Schismatoglottis giamensis* S.Y.Wong & Y.C.Hoe sp. nov.

Type. Malaysia, Sarawak, Kuching Division, Siburan, Kampung Giam, 01° 19' 16.1"; 110° 16' 16.7", 20 June 2009, P.C.Boyce & S.Y.Wong Ar2549 (Holotype SAR) (**Figure 3.13 & 3.14**).

Diagnosis

Schismatoglottis giamensis S.Y.Wong & Y.C.Hoe is most similar to *S. roh* by having stigma larger than ovary and staminodes at interstice are similar to interpistillar staminodes. However, *S. giamensis* differs by having weakly channeled petiole till ca ½ its length and interstice is partially naked with two whorls of staminodes distally.

Medium to moderately robust, evergreen, stoloniferous **herb** forming clumps, 30 – 80 cm tall. **Stems** hypogeal, hapaxanthic, 1 – 2 cm diam. **Leaves** 3 – 5 together; **petiole** terete, smooth, 50 – 62 cm long, green, weakly channeled ca ½ in its length, longitudinal striate prominent distally, darker green; **petiolar sheath** 16 – 20 cm long x 5 – 10 mm wide, sheathing for ca $\frac{1}{3}$ of petiole length, persistent, membranous, fully attached with a very short ligule, equal at both sides, slightly inrolled or sometimes straight, tapering, scattering longitudinal green striates visible; **blades** mostly ovato-sagittate to ovato-cordate (sometimes oblong-lanceolate with the base cordate), 18 – 33 cm long x 8 – 18 cm wide, softly coriaceous, adaxially glossy green, abaxially paler, posterior lobes subtriangular, 6.5 – 10 cm, sinus 9 – 11 cm across, apex acute for 2 – 3 cm, ultimately mucronate for 7 – 12 mm; **midrib** adaxially flush with blade, raised

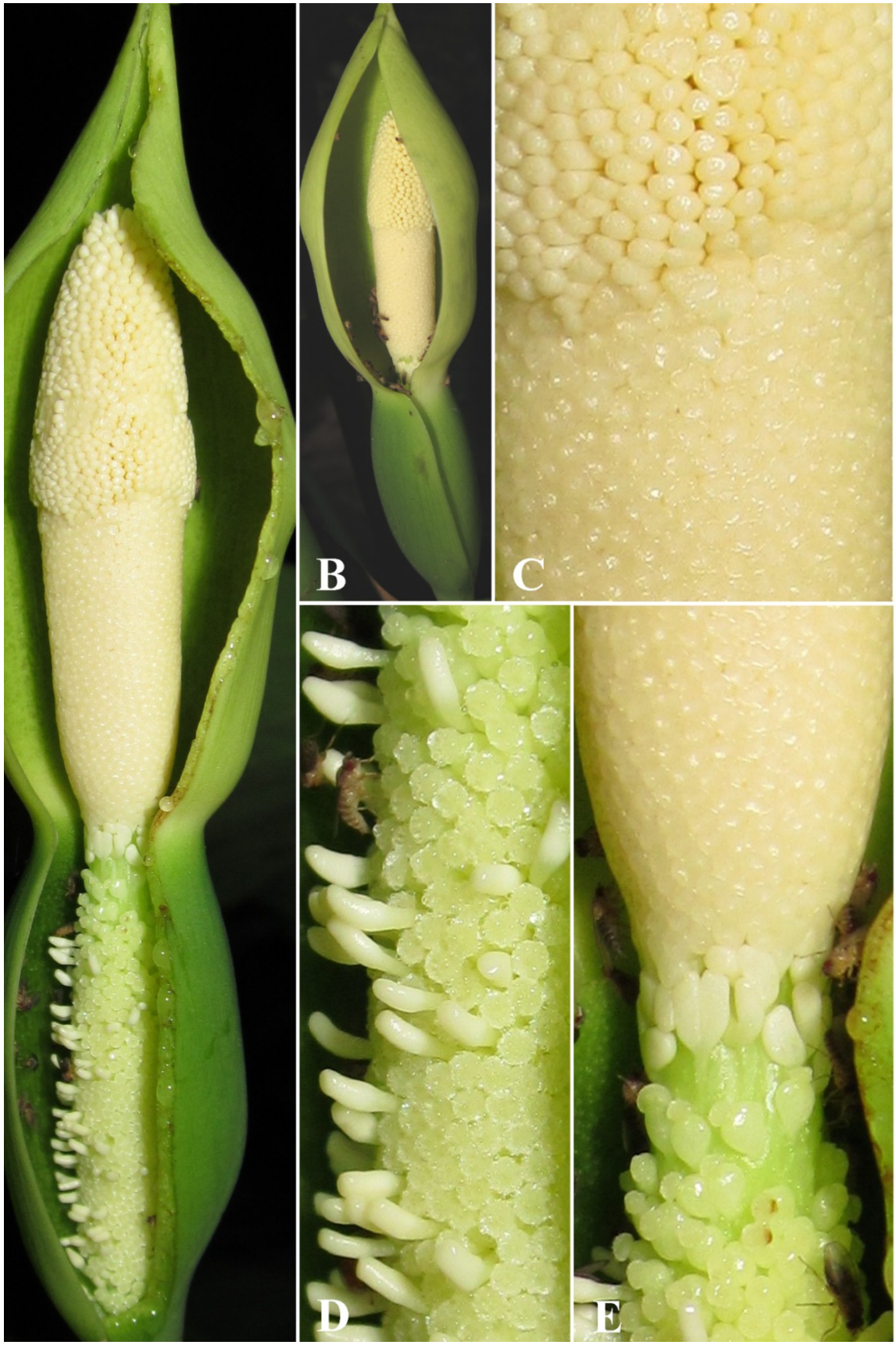

Figure 3.13. *Schismatoglottis giamensis* S.Y.Wong & Y.C.Hoe. A. Inflorescence spadix; B. Inflorescence; C. Appendix and staminate flower zone; D. Pistillate flower zone; E. Interstice.

Figure 3.14. *Schismatoglottis giamensis* S.Y.Wong & Y.C.Hoe. A. Plant in habitat; B. Abaxial blade; C. Adaxial blade; D. Petiolar sheath and inflorescence bud; E. Scattered longitudinal medium green striate on petiole.

abaxially, ca 5 mm wide at the insertion; **primary lateral veins** ca 17 per side, diverging at 30° – 80° from the midrib, raised adaxially towards the midrib, marginally impressed, entirely raised abaxially; **interprimary veins** raised adaxially, alternating irregularly with primaries; few (0 – 2) **secondary veins** raised from each primary vein (3 – 4 raised from primary veins near to insertion); **tertiary veins** inconspicuous; **vein-like pellucid glands** slightly visible. **Inflorescences** up to 3, erect, emit esteric acid-like smell during pistillate anthesis, absent during staminate anthesis; **peduncle** 5 – 15 cm long x 5 – 9 mm wide, terete, green, erect at anthesis; **spathe** 9 – 11 cm long; **lower spathe** narrowly ovoid, 3.5 – 5 cm long x ca 2 cm wide, green, longitudinally ridged, separated from spathe limb by a constriction coinciding with the lower staminate flower zone; **spathe limb** turbinate, 5.5 – 6.5 cm long x ca 2.9 cm wide, mucronate for ca 3 mm, pale greenish yellow at pistillate anthesis, pallid prior to staminate anthesis, falls fresh in a single piece at the onset of staminate anthesis; **spadix** ca 9 cm long, shorter than spathe, sessile; **pistillate flower zone** tapering cylindric, ca 4.2 cm long × ca 1.1 cm wide, ca $\frac{2}{5}$ length of spadix, apex slightly tapering, light green; **pistils** sub-cylindric to sub-globose, ca 1 mm long x 0.4 mm wide, densely arranged at base, laxly arranged and impressed at apex of pistillate flower zone; **style** barely differentiated; **stigma** globose from above, truncated, weakly wider than ovary, ca 0.5 mm diam., wet with stigmatic secretion at the onset of pistillate anthesis; **interpistillar staminodes** clavate, stipe hardly differentiated, 86 – 158, 0.5 – 0.8 mm in diam., ca double the height of pistils, scattering, white; **interstice** sub-cylindric, 5 – 10 mm long x 5 – 6 mm wide, slender than pistillate and staminate flower zone, partially naked, comprised ca 2 whorls of flattened clavate staminodes that resemble interpistillar staminodes (flattened), intergrading into the lower staminate flower zone, pistillodes flattened at proximal; **staminate flower zone** sub-cylindric, narrower at proximal but wider at distal, ca 2 cm long × 8 – 11 mm wide, ca $\frac{2}{9}$ length of spadix, yellowish

white; **staminate flowers** ca 1 mm long x ca 0.8 mm wide, bow tie shaped from above, densely arranged, each comprising 2 truncate stamens, overtopping by a broad, flat/raised connective; **pollen** powdery, white; **appendix** bullet-shaped, ca 2 cm long x ca 1 cm wide, ca $\frac{2}{9}$ length of spadix, base weakly (ca 0.2 mm) wider than apex of staminate flower zone, yellowish white; **staminodes** sub-globose, rather sub-columnar and weakly protruded towards the tip of appendix, ca 1 mm long x 0.5 – 0.8 mm wide, densely arranged. **Infructescences** 1 – 3, 4.5 – 5 cm long x ca 2 cm wide, declinate; **lower spathe** entirely persistent, reflex when ripe; **fruits** ca 2 mm long x 1 – 2 mm wide, green to yellow; **seeds** ovoid ellipsoid, ca 0.5 mm diam., 3 – 17 per fruit, encased with greenish yellow gel.

Distribution – *Schismatoglottis giamensis* is known from the type locality and its vicinity.

Ecology – Perhumid lowland tropical forests which are adjacent to karst limestones, 70 m asl.

Etymology – The specific epithet is derived from the locality, Kampung (Village) Giam.

Other specimens seen – Sarawak, Kuching Division, Padawan, Kampung Danu, riverside, 01.16 N 110. E, 45 m asl, 5 October 2014, S.Y. Wong & P.C. Boyce Ar4992 (SAR); Kampung Danu, Gua Tut entrance, 01.17 N 110.15 E, 50 m asl, 5 October 2014*, S.Y.Wong & P.C.Boyce Ar4995* (SAR).

3.4.7 Description of *Schismatoglottis adducta* S.Y.Wong & Y.C.Hoe sp. nov.

Type. Malaysia, Sarawak, Sri Aman Division, Lubok Antu, Engkilili, Tempat Rekreasi Sungai Raya, 01° 06' 49.2"; 111° 30' 56.8", 9 December 2005, P.C.Boyce, Jeland ak Kisai, Jipom ak Tisai & Mael ak Late Ar1632 (Holotype SAR) (**Figure 3.15 & 3.16**).

Diagnosis

Schismatoglottis adducta S.Y.Wong & Y.C.Hoe is very distinctive with the spadix which gives the impression having being stretched, leaving the middle part, slender and attenuated. The interstice comprises of flattened pistillodes and staminodes.

Medium to moderately robust, evergreen, stoloniferous **herb** forming clumps, 35 – 60 cm tall. **Stems** hypogeal, hapaxanthic, 1 – 1.5 cm diam. **Leaves** 3 – 5 together; **petiole** D-shaped, smooth, 20 – 35 cm long, green, channeled throughout its length, longitudinal striates prominent distally, darker green; **petiolar sheath** 6 – 10 cm long x 5 – 8 mm wide, sheathing for $\frac{1}{3}$ of petiole length, persistent, membranous, fully attached with a very short ligule, equal at both sides, slightly inrolled or sometimes straight, tapering, green with scattered greenish dotting striate; **blades** ovato-sagittate to ovato-cordate (sometimes oblong-lanceolate with the base cordate), 18 – 33 cm long x 10 – 18 cm wide, softly coriaceous, adaxially glossy green, some variegated with yellowish bands, abaxially paler, posterior lobes subtriangular for 3.5 – 7.5 cm, sinus 5.5 – 7 cm across, apex acuminate to acute for 1 – 2 cm, ultimately mucronatefor 5 – 11 mm; **midrib** adaxially flush with blade, raised abaxially, 5 – 6 mm at the insertion; **primary lateral veins** ca 17 per side, diverging at 30° – 80° from the midrib, raised adaxially towards the midrib, marginally impressed, entirely raised abaxially; **interprimary veins** raised adaxially; few (0 – 2) **secondary veins** raised each primary veins (3 – 4 raised

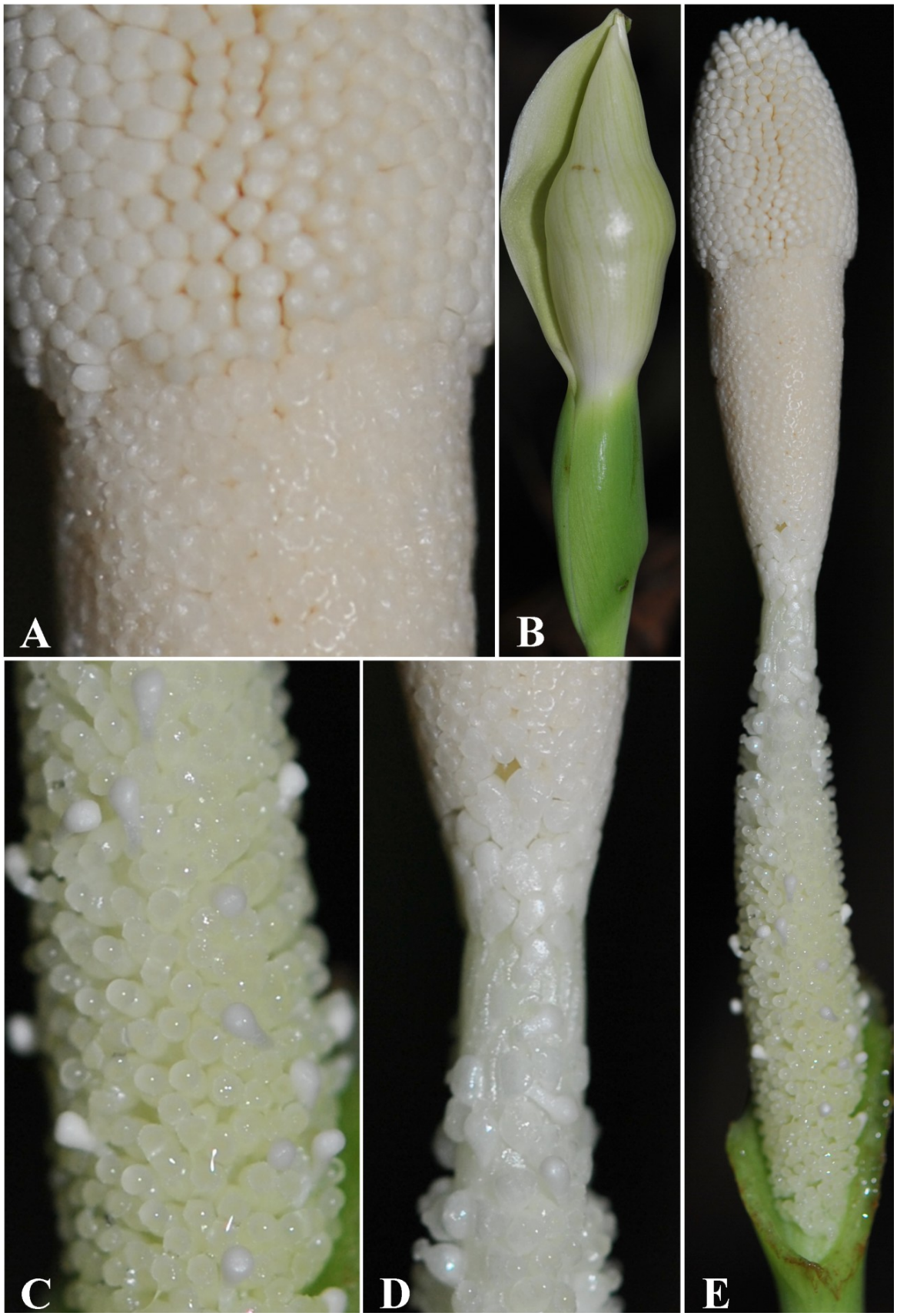

Figure 3.15. *Schismatoglottis adducta* S.Y.Wong & Y.C.Hoe. **A.** Appendix and staminate flower zone; **B.** Inflorescence; **C.** Pistillate flower zone; **D.** Interstice; **E.** Inflorescence spadix.

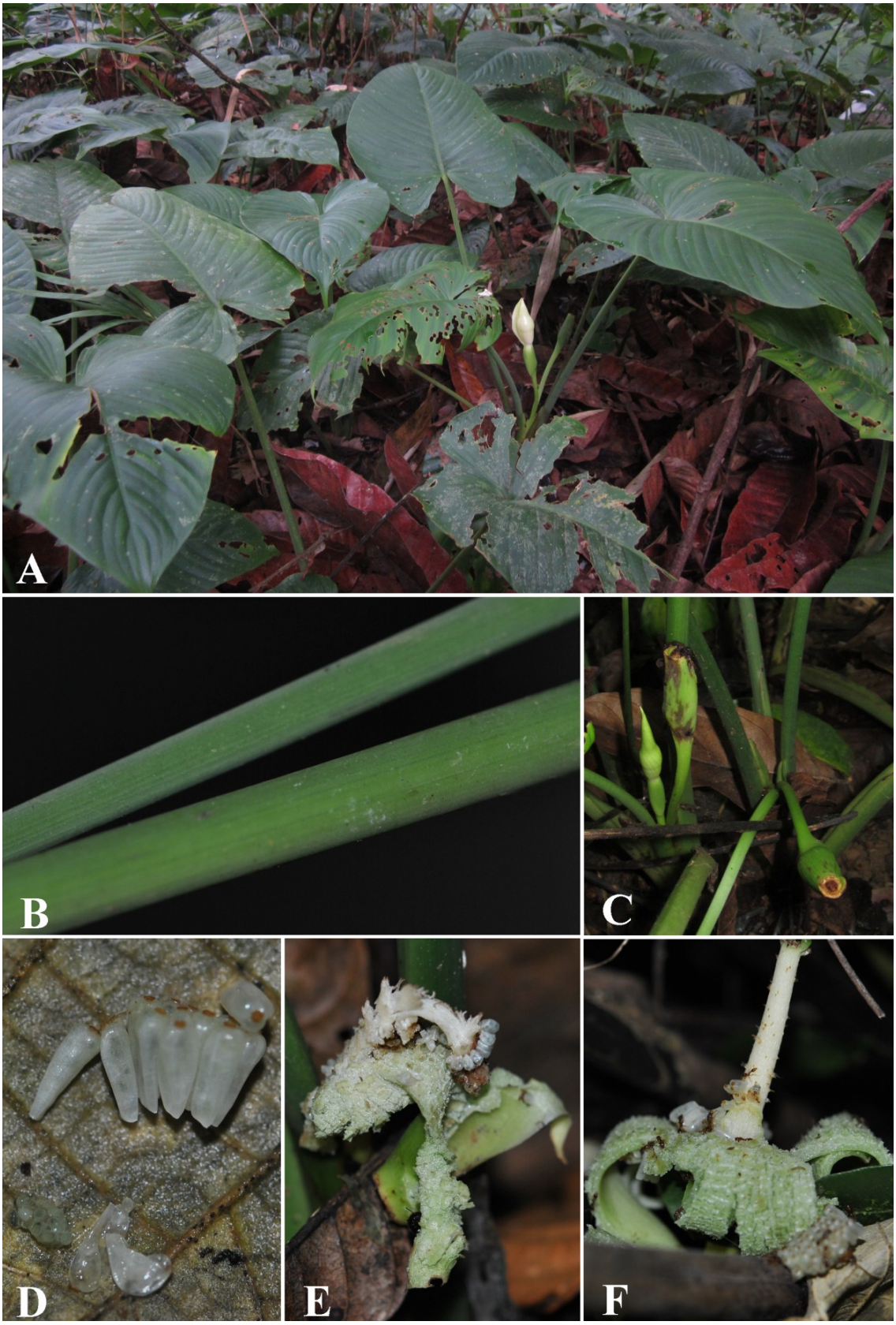

Figure 3.16. *Schismatoglottis adducta* S.Y.Wong & Y.C.Hoe. **A.** Plant in habitat; **B.** Scattered longitudinal medium green striate on petiole; **C.** Synflorescence; **D.** Fruits; **E & F.** Spathe infructescence splitting basiscopically (E) and acropetally (F).

from primary veins near insertion); **tertiary veins** inconspicuous; **vein-like pellucid glands** slightly visible. **Inflorescences** up to 3, erect, emit esteric acid-like smell during pistillate anthesis, absent during staminate anthesis; **peduncle** 5 – 13 cm long x 5 – 10 mm wide, terete, green, erect at anthesis; **spathe** ca 8.5 cm long; **lower spathe** narrowly ovoid, ca 3.2 cm long x ca 1.3 cm wide, green, longitudinally ridged, separated from spathe limb by a constriction coinciding with the lower staminate flower zone; **spathe limb** turbinate, ca 5 cm long x ca 2.2 cm wide, mucronate for ca 2 mm, pale greenish yellow at pistillate anthesis, pallid prior to staminate anthesis, falls fresh in a single piece at onset of staminate anthesis; **spadix** 5.5 – 6.8 cm long, shorter than spathe, sessile; **pistillate flower zone** tapering, 2.6 – 3 cm long × 6 – 8 mm wide, ca $\frac{1}{2}$ length of spadix, light yellow; **pistils** sub-cylindric to sub-globose, 0.8 – 1 mm in diam., densely arranged; **style** barely differentiated; **stigma** globose from above, truncated, slightly smaller than ovary, ca 0.3 mm diam., wet with stigmatic secretion at the onset of pistillate anthesis; **interpistillar staminodes** clavate, stipe slender 32 – 66, ca 0.5 mm in diam., up to double the height of pistils, scattering, flatten distally, white; **interstice** sub-cylindric, 3 – 7 mm long x 3 – 4 mm wide, slender than pistillate and staminate flower zone, partially naked, comprised 2 – 3 whorls of flattened disc – like staminodes, intergrading into the lower staminate flower zone, pistillodes flattened at proximal; **staminate flower zone** bullet-shaped, strongly tapering basally, ca 1.6 cm long × 7 – 8 mm wide, ca $\frac{1}{4}$ length of spadix, yellowish white; **staminate flowers** ca 1 mm long x ca 0.5 mm wide, bow tie shaped from above, densely arranged, each comprising 2 truncate stamens, sunken in, separated by a narrow connective, densely arranged; **pollen** powdery, white; **appendix** bullet-shaped, 1 – 1.5 cm long x 8 – 9 mm wide, ca $\frac{1}{6}$ length of spadix, base weakly (ca 0.3 mm) wider than apex of staminate flower zone, yellowish white; **staminodes** sub-globose, sub-columnar towards the appendix, ca 2 mm long x ca 0.5 mm wide, densely arranged. **Infructescences** 1 – 3, 4.4 – 4.6

cm long x 1.1 – 1.3 cm wide, declinate; **lower spathe** entirely persistent, reflex when ripe; **fruits** ca 3 mm long x ca 2 mm wide, light yellow; **seeds** ovoid ellipsoid, ca 0.4 mm diam., 4 – 13 per fruit, encased with greenish yellow gel.

Distribution – *Schismatoglottis adducta* is known from its locality at Tempat Rekreasi Sungai Raya, Sri Aman Division, Sarawak and its vicinity.

Ecology – Perhumid lowland tropical forest, shale, occurring along the alluvial stream margin 13 m asl.

Etymology – The specific epithet is derived from the Latin suffix, *'adductus'*, in allusion to the spadix which gives the impression having being stretched, leaving the middle part, slender and attenuated.

Other specimens seen – Sarawak, Sri Aman Division, Lubok Antu, Engkilili, Tempat Rekreasi Sungai Raya, 01° 06' 49.2"; 111° 30' 56.8", 9 December 2005, *P.C.Boyce, Jeland ak Kisai, Jipom ak Tisai & Mael ak Late Ar5077* (SAR); Lubok Antu, Engkilili, Tempat Rekreasi Sungai Raya, 01° 06' 49.2"; 111° 30' 56.8", 9 December 2005, *P.C.Boyce, Jeland ak Kisai, Jipom ak Tisai & Mael ak Late Ar5078* (SAR).

3.4.8 Description of *Schismatoglottis pantiensis* S.Y.Wong & Y.C.Hoe sp. nov.

Type. Malaysia, Johor Bahru, Kota Tinggi, Hutan Simpan Panti, 01°48.595' 103°51.099', 4 December 2013, Y.C.Hoe Ar4322 (Holotype SAR) (**Figure 3.17 & 3.18**).

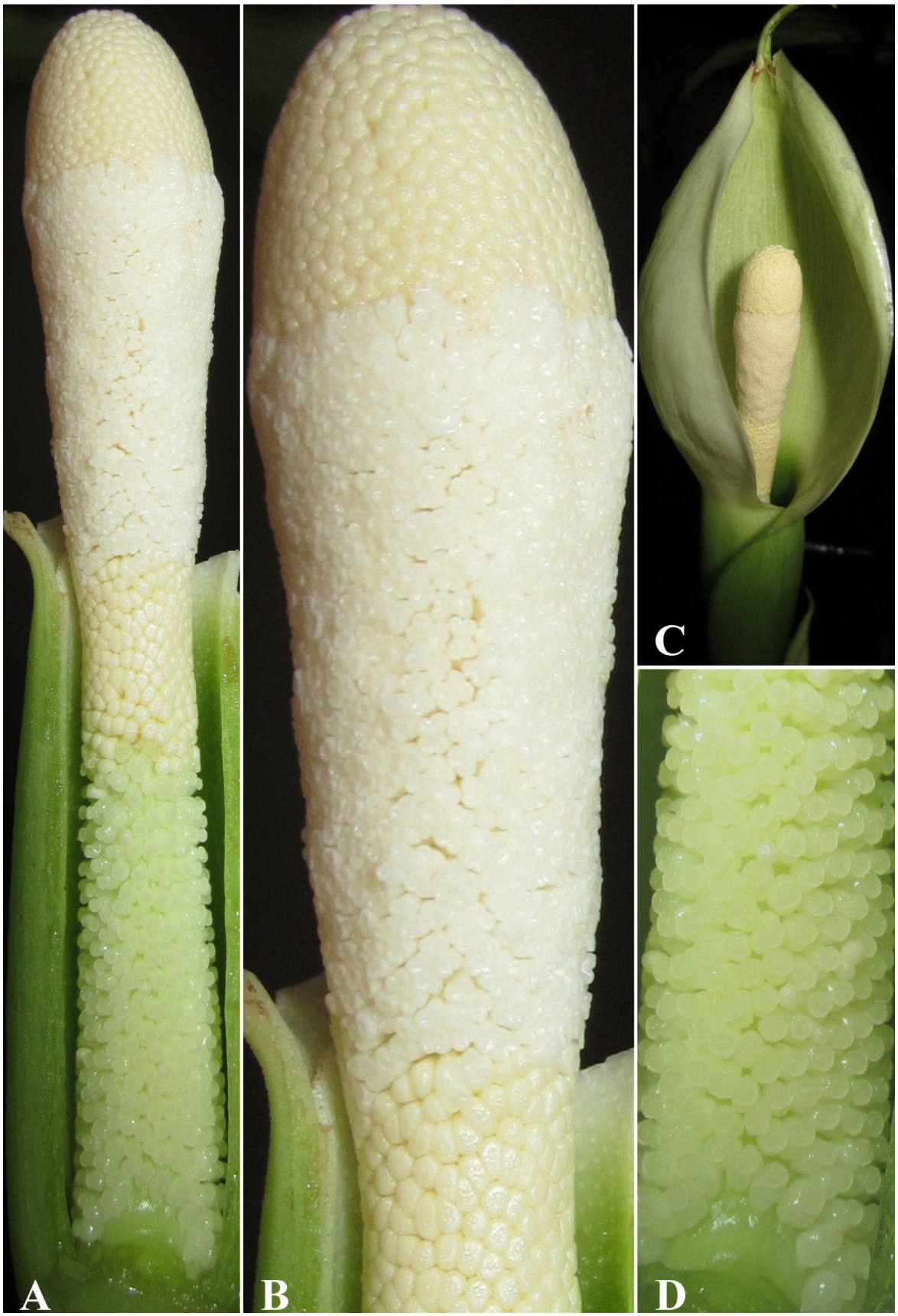

Figure 3.17. *Schismatoglottis pantiensis* S.Y.Wong & Y.C.Hoe. **A.** Inflorescence spadix; **B.** Appendix and staminate flower zone; **C.** inflorescence; **D.** Pistillate flower zone.

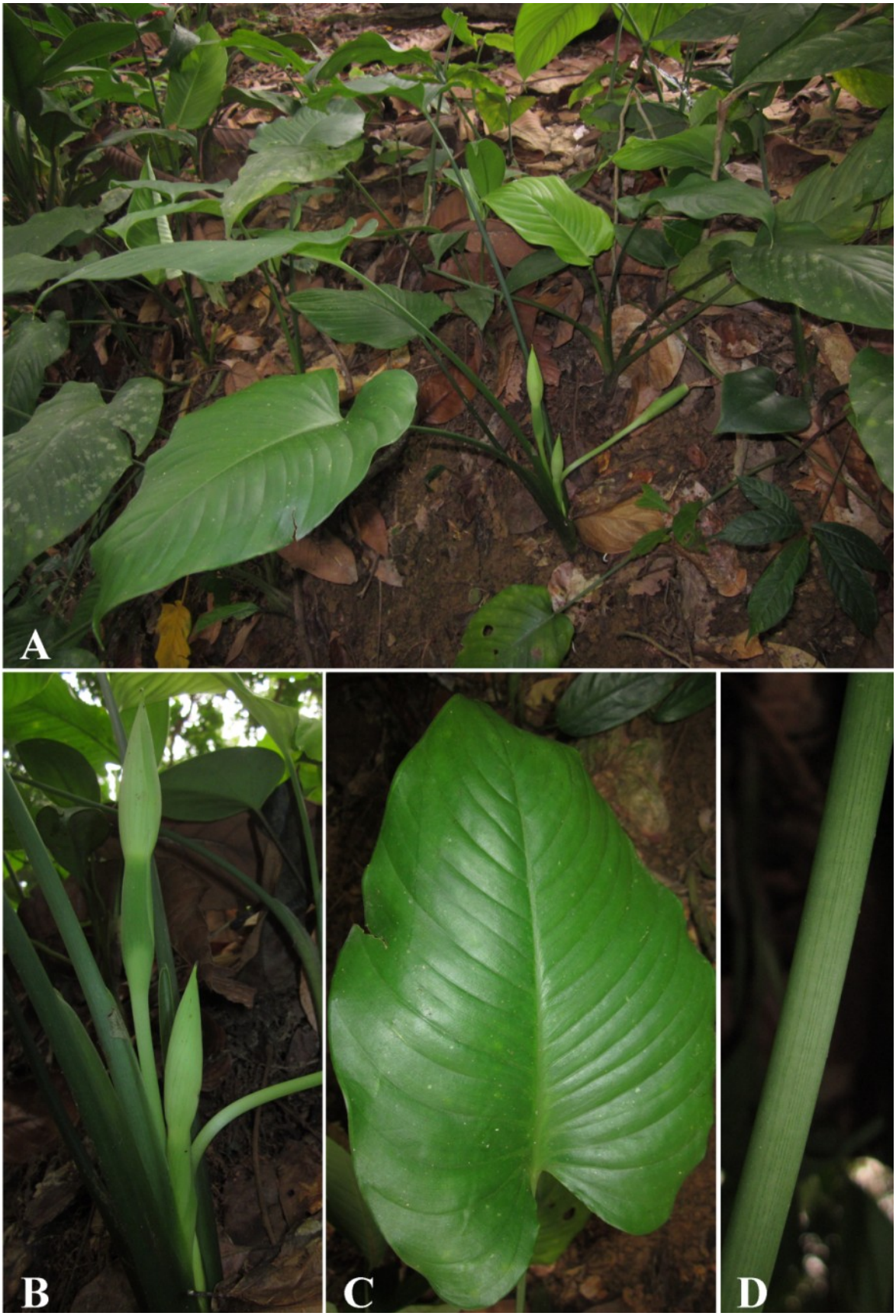

Figure 3.18. *Schismatoglottis pantiensis* S.Y.Wong & Y.C.Hoe. **A.** Plant in habitat; **B.** Synflorescence; **C.** Adaxial blade; **D.** Longitudinal medium green ridge on the petiole.

Diagnosis

Schismatoglottis pantiensis S.Y.Wong & Y.C.Hoe is most similar to *S. caesia* by having D-shaped petiole, weakly channelled petiole throughout its length, absence of vein-like pellucid glands, interpistillar staminodes few, and equal or slightly taller than pistils but differs by having interstice with only staminodes (which resemble staminodes at appendix) and apex of staminate flower zone is rather equalling with the base of appendix.

Medium to moderately robust, evergreen, stoloniferous **herb** forming clumps, 35 – 40 cm tall. **Stems** hypogeal, hapaxanthic, 1 – 1.5 cm diam. **Leaves** 3 – 7 together; **petiole** D-shaped, smooth, 15 – 18 cm long, green, weakly channeled throughout its length, longitudinal striates prominent distally; **petiolar sheath**, ca 8 cm long x ca 0.5 wide, sheathing for $\frac{1}{3} - \frac{1}{2}$ of petiole length, persistent, membranous, fully attached with a very short ligule, equal at both sides, slightly inrolled or sometimes straight, tapering, green with scattered greenish dotting striate; **blades** ovato-sagittate to ovato-cordate (sometimes oblong-lanceolate with the base cordate), 16 – 23 cm long x 9 – 13 cm wide, softly coriaceous, adaxially glossy green, abaxially paler, posterior lobes subtriangular for 4 – 6 cm, sinus 4.5 – 5 cm across, apex acute for ca 2 cm, ultimately mucronate for ca 5 mm; **midrib** adaxially flush with blade, raised abaxially, ca 4 mm at the insertion; **primary lateral veins** ca 14 per side, diverging at 30° – 80° from the midrib, raised adaxially towards the midrib, marginally impressed, entirely raised abaxially; **interprimary veins** raised adaxially, alternating irregularly with primaries; secondary veins raised from each primary veins rather inconspicious (1 – 3 weakly raised from primary veins near to insertion); **tertiary veins** inconspicuous; **vein-like pellucid glands** not visible. **Inflorescences** up to 3, erect, emit esteric acid-like smell during pistillate anthesis, absent during staminate anthesis; **peduncle** ca 13 cm long x ca 6 mm wide, terete, green, erect at

anthesis; **spathe** ca 12 cm long; **lower spathe** narrowly ovoid, ca 5 cm long x ca 1.8 cm wide, green, longitudinally ridged, separated from spathe limb by a constriction coinciding with the lower staminate flower zone; **spathe limb** turbinate, ca 7 cm long x ca 2.7 cm wide, mucronate for ca 4 mm, pale greenish yellow at pistillate anthesis, slightly pallid prior to staminate anthesis, split acropetally, caducous and falling in a single piece at onset of staminate anthesis; **spadix** ca 7 cm long, shorter than spathe, sessile; **pistillate flower zone** cylindric**,** ca 3.5 cm long × ca 7 mm wide, ca $\frac{1}{2}$ length of spadix, light green; **pistils** sub-globose, ca 1 mm long x 0.5 – 1.2 mm wide, densely arranged; **style** slightly naked, 4 – 5 mm long, light green; **stigma** sub-globose from above, truncated, smaller than ovary, ca 0.3 mm diam., wet with stigmatic secretion at the onset of pistillate anthesis; **interpistillar staminodes** clavate, stipe stout, 42 – 56, ca 0.5 mm in diam., similar height with pistils, scattering, white; **interstice** cylindric, 0.8 – 1 cm long x ca 6 mm wide, cylindric, weakly slender than pistillate and staminate flower zone, not naked, comprised 10 – 12 whorls of sub-globose staminodes that resemble staminodes at appendix, densely packed, staminodes and pistillodes not impressed; **staminodes** 0.5 – 1 mm wide, sub-globose, light yellow; **staminate flower zone** sub-cylindric, narrower at proximal but wider at distal, ca 1.7 cm long × 7 – 9 mm wide, ca $\frac{1}{4}$ length of spadix, yellowish white; **staminate flowers** bow tie shaped from above, ca 1 mm long x ca 0.5 mm wide, densely arranged, each comprising 2 truncate stamens, sunken in, separated by a narrow connective, densely arranged; **pollen** powdery, and white; **appendix** bullet-shaped, ca 0.9 cm long x ca 0.9 cm wide, ca $\frac{1}{7}$ length of spadix, base rather equaling apex of staminate flower zone, yellowish white; **staminodes** sub-globose, ca 1 mm diam, densely arranged. **Infructescences** 1 – 3, 3.2 – 5.2 cm long x 0.8 – 1.5 cm wide, declinate; **lower spathe** entirely persistent, reflex when ripe; **fruits** ca 2 mm long x 1 – 1.8

mm wide, light green; **seeds** ovoid ellipsoid, ca 0.4 mm diam., 4 – 17 per fruit, encased with greenish yellow gel.

Distribution – *Schismatoglottis pantiensis* is only known from its locality at the hiking trail to Hutan Simpan Panti, Kota Tinggi, Johor.

Ecology – Lowland tropical forest, sandstones, terrestrial along the margin of the waterfall stream, 14 m asl.

Etymology – The specific epithet is derived from the name of the type locality plus the Latin suffix, *–ensis*, to indicate coming from.

Other specimens seen – Malaysia, Johor Bahru, Kota Tinggi, Hutan Simpan Panti, 01°48.595' 103°51.099', 4 December 2013, *Y.C.Hoe Ar4323* (SAR); Kota Tinggi, Hutan Simpan Panti, 01°48.595' 103°51.099', 4 December 2013, *Y.C.Hoe Ar4324* (SAR); Kota Tinggi, Hutan Simpan Panti, 01°48.595' 103°51.099', 4 December 2013, *Y.C.Hoe Ar4325* (SAR); Kota Tinggi, Hutan Simpan Panti, 01°48.595' 103°51.099', 4 December 2013, *Y.C.Hoe Ar4337* (SAR).

3.4.9 Description of *Schismatoglottis baangongensis* S.Y.Wong & Y.C.Hoe sp. nov.

Type: Malaysia, Sarawak, Kuching Division, Padawan, Siburan, Kampung Sikog, trail to Baan Gong water fall, 01° 20' 16.1"; 110° 20' 09.6", 26 July 2009, P.C.Boyce & S.Y.Wong Ar2588 (Holotype SAR) (**Figure 3.19 & 3.20**).

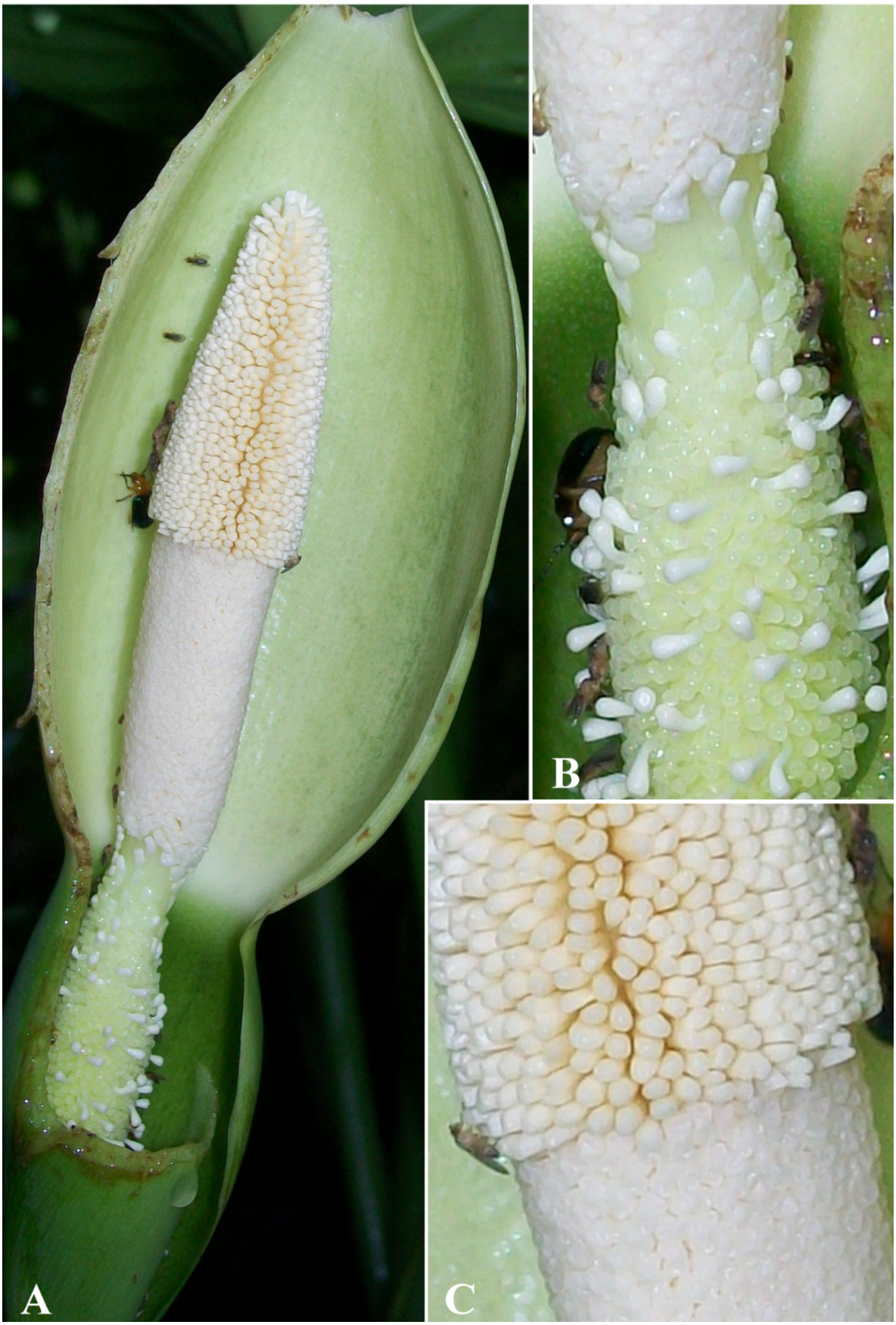

Figure 3.19. *Schismatoglottis baangongensis* S.Y.Wong & Y.C.Hoe. **A.** Inflorescence spadix; **B.** Interstice and pistillate flower zone; **C.** Appendix and staminate flower zone.

Figure 3.20. *Schismatoglottis baangongensis* S.Y.Wong & Y.C.Hoe. **A.** Plants in habitat; **B.** Synflorescence; **C.** Plant in habitat; **D.** Inflorescence.

Diagnosis

Schismatoglottis baangongensis S.Y.Wong & Y.C.Hoe is most similar to *S. giamensis* by the proportion of the pistillate flower zone is ca $^{2}/_{5}$ of the spadix but is readily distinguished by the stigma smaller than ovary, constriction at interstice, and long bullet shaped appendix with the slender clavate staminodes which are laxly arranged.

Medium to moderately robust, evergreen, stoloniferous **herb** forming clumps, 30 – 90 cm tall. **Stems** hypogeal, hapaxanthic, 0.5 – 1.5 cm diam. **Leaves** 3 – 5 together; **petiole** terete, smooth, 42 – 48 cm long, green, weakly channeled ca 1/5 in its length, longitudinal striates prominent distally, darker green; **petiolar sheath** 11 – 14 cm long x 5 – 10 mm wide, up to $^{3}/_{10}$ of petiole length, persistent, membranous, fully attached with a very short ligule, equal at both sides, slightly inrolled or sometimes straight, tapering, longitudinal striates visible; **blades** ovato-sagittate to ovato-cordate (sometimes oblong-lanceolate with the base cordate), 20 – 39 cm long x 13 – 23 cm wide, softly coriaceous, adaxially glossy green, abaxially paler, posterior lobes subtriangular, 7 – 11 cm, sinus 8 – 11 cm across, apex acuminate to acute for ca 2 cm, ultimately mucronate for ca 1 cm; **midrib** adaxially flush with blade, raised abaxially, ca 5 mm wide at the insertion; **primary lateral veins** ca 14 per side, diverging at 30° – 80° from midrib, raised adaxially towards the midrib, marginally impressed, entirely raised abaxially; **interprimary veins** raised adaxially, alternating irregularly with primaries; few (0 – 2) **secondary veins** raised from each primary vein (3 – 4 secondary veins raised from primary veins near to insertion); **tertiary veins** inconspicuous; **vein-like pellucid glands** slightly visible. **Inflorescences** up to 4, erect, emit esteric acid-like smell during pistillate anthesis, absent during staminate anthesis; **peduncle** 10 – 15 cm long x 4 – 9 mm wide, terete, green, erect at anthesis; **spathe** 11 – 12.5 cm long; **lower spathe** narrowly ovoid, ca 4 cm

long x ca 2.3 cm wide, green, longitudinally ridged, separated from spathe limb by a constriction coinciding with interstice; **spathe limb** turbinate, ca 6.5 cm long x ca 3.3 cm wide, mucronate for ca 2 mm, pale yellowish green at pistillate anthesis, pallid prior to staminate anthesis, falls fresh in a single piece at the onset of staminate anthesis; **spadix** 9 – 10.5 cm long, shorter than spathe, sessile; **pistillate flower zone** slender obconic, 4 – 5 cm long × ca 1 cm wide, ca $^{2}/_{5}$ of spadix length, light green; **pistils** sub-cylindric to sub-globose, 0.8 – 1.2 mm in diam., densely arranged; **style** barely differentiated; **stigma** globose from above, truncated, smaller than ovary, ca 0.3 mm diam.; **interpistillar staminodes** clavate, stipe slender, 101 – 150, 0.5 – 0.8 mm in diam., double the height of pistils, white, scattering; **interstice** cylindric, 0.5 – 1 cm long x 5 – 6.5 mm wide, slender than pistillate and staminate flower zone, partially naked, flattened trapezoid staminodes at proximal and distal, pistillodes flattened at proximal; **staminate flower zone** sub-cylindric, narrower at proximal but wider at distal, 2.2 – 2.7 cm long × 9 – 12 mm wide, ca $^{3}/_{10}$ length of spadix, yellowish white; **staminate flowers** ca 1 mm long x ca 0.5 mm wide, bow tie shaped from above, each comprising 2 truncate stamens, densely arranged, with the thecae sunken, separated by a narrow, raised connective; **pollen** powdery, white; **appendix**, long bullet shaped, 2.2 – 2.5 cm long x ca 1 cm wide, ca $^{3}/_{10}$ length of spadix, base wider (ca 1 mm) than apex of staminate flower zone, creamy yellow; **staminodes** slender clavate, ca 2 mm long x ca 0.7 mm wide, somewhat laxly arranged, creamy yellow. **Infructescences** 1 – 4, 4 – 6 cm long x 2 – 2.2 cm wide, declinate; **lower spathe** entirely persistent, reflex when ripe; **fruits** 2 – 4 mm long x 1 – 2.5 mm wide, green to yellow; **seeds** ovoid ellipsoid, ca 0.4 mm diam., 13 – 22 per fruit, encased with greenish yellow gel.

Distribution – *Schismatoglottis baangongensis* is known from the type locality and its vicinity.

Ecology – Perhumid lowland tropical forests which are adjacent to karst limestones, occurring along the trails next to small streams, 70 – 75 m asl.

Etymology – Derived from the name of the type locality plus the Latin suffix, *–ensis*, to indicate coming from.

Other specimens seen – Malaysia, Sarawak, Kuching Division, Padawan, Siburan, Kampung Sikog, trail to Baan Gong water fall, 01° 20' 16.1"; 110° 20' 09.6", 26 July 2009*, P.C.Boyce & S.Y.Wong Ar2587* (SAR).

3.4.10 Description of *Schismatoglottis pseudoniahensis* S.Y.Wong & Y.C.Hoe sp. nov.

Malaysia, Sarawak, Miri, Niah Suai, Niah N. P., 03 49 N 113 45 E, 30 March 2014, Y.C.Hoe Ar4666 (SAR) (**Figure 3.21 & 3.22**).

Diagnosis

Schismatoglottis pseudoniahensis S.Y.Wong & Y.C.Hoe is similar to *S. calyptrata* by having densely massed staminate flowers with individual flowers not discernible. However, leaf blade is softly coriaceous in *S. pseudoniahensis* but leathery in *S. calyptrata*, vein like pellucid glands are present in *S. pseudoniahensis* but absent in *S. calyptrata*, interpistillar staminodes are only ½ taller than pistils in *S. pseudoniahensis* but double the height of pistils in *S. calyptrata.*

Medium to moderately robust **herb**, evergreen, forming clumps, 50 – 85 cm tall. **Stems** pleionanthic, ca 2 cm diam. **Leaves** 3 – 6 together; **petiole** D-shaped, smooth, 27 – 43 cm long, green, channeled entirely in its length, a few longitudinal striates, darker green; **petiolar**

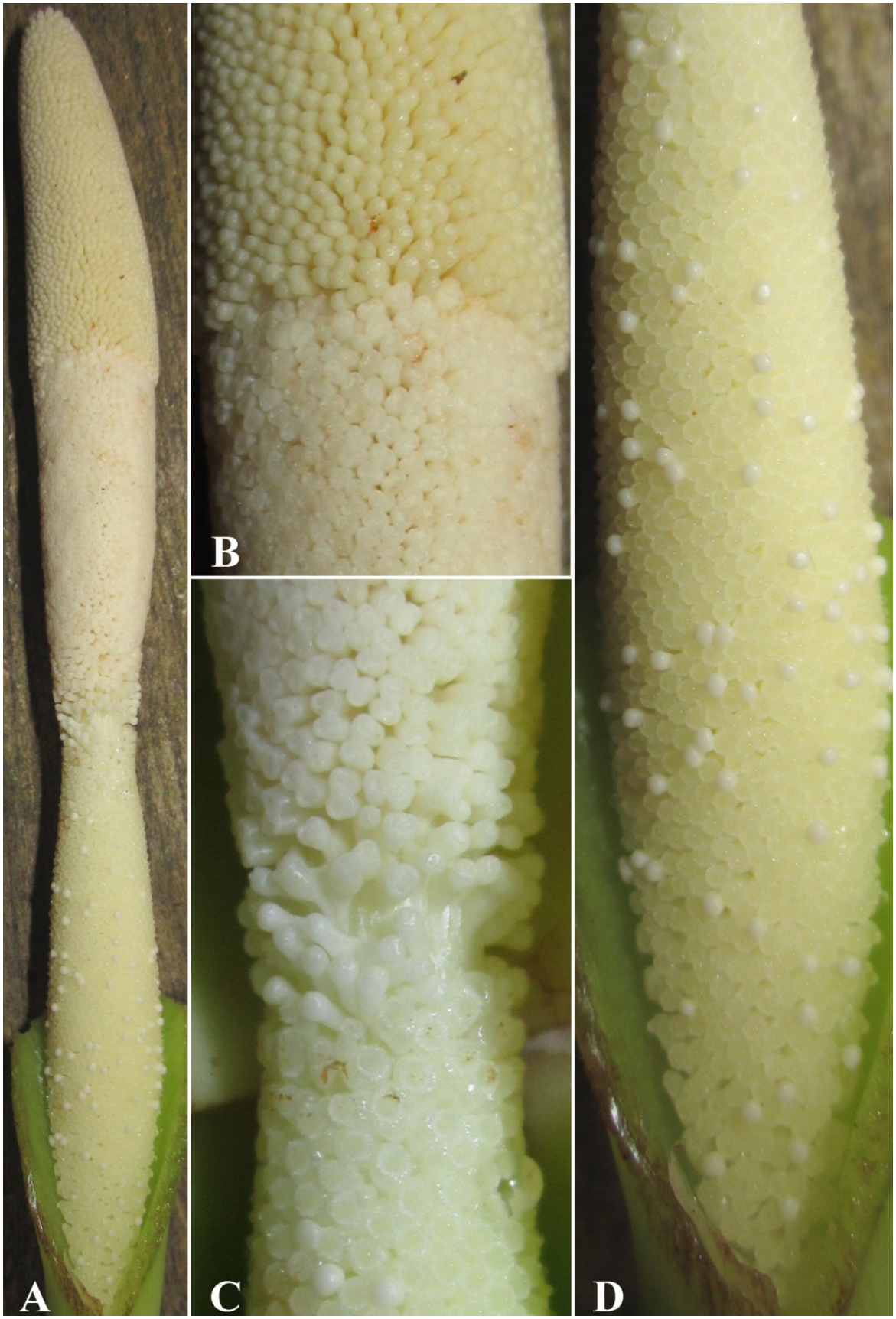

Figure 3.21. *Schismatoglottis pseudoniahensis* S.Y.Wong & Y.C.Hoe. **A.** Inflorescence spadix; **B.** Appendix and staminate flower zone; **C.** Interstice; **D.** Pistillate flower zone.

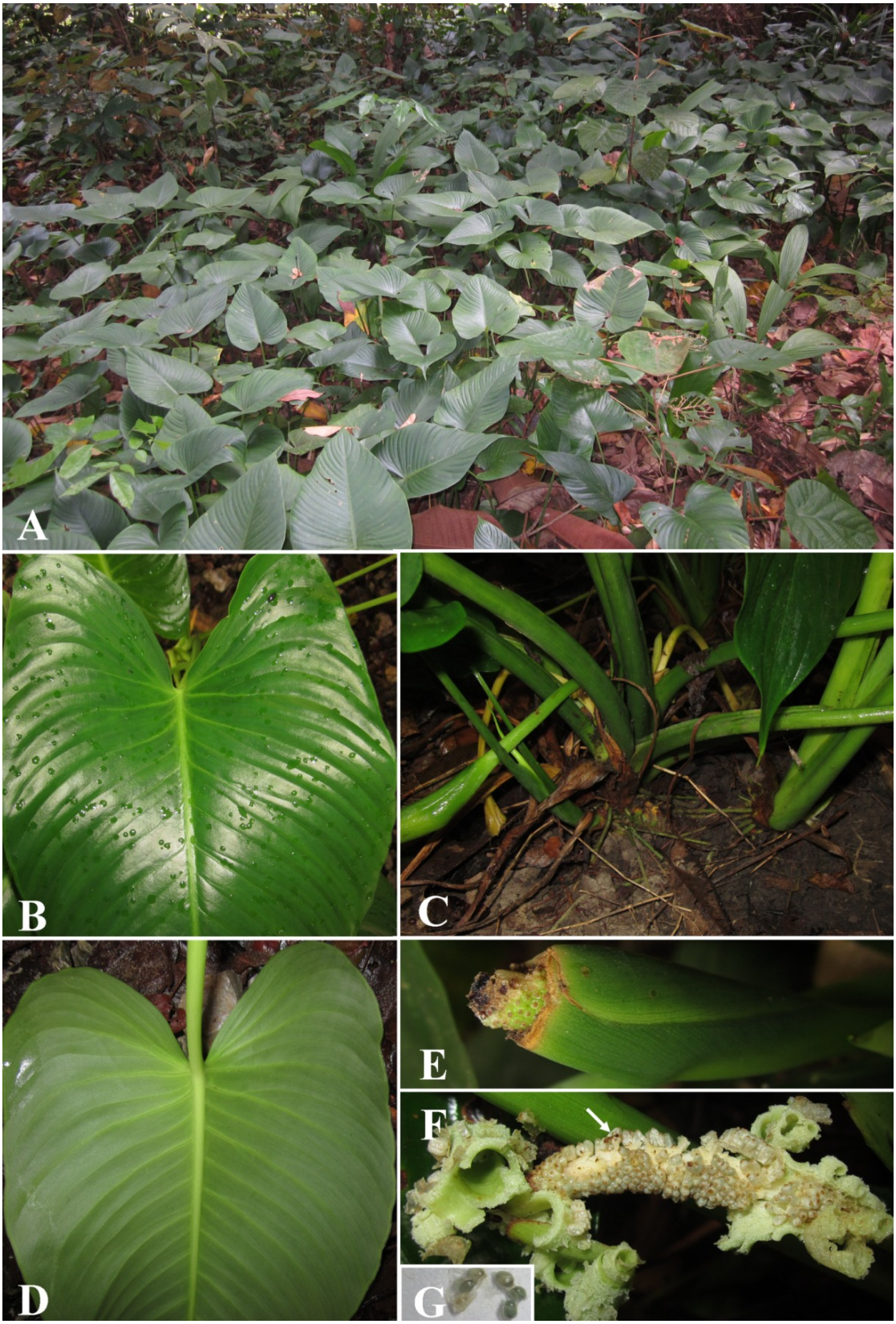

Figure 3.22. *Schismatoglottis pseudoniahensis* S.Y.Wong & Y.C.Hoe. **A.** Plant in habitat; **B.** Adaxial blade; **C.** Hapaxanthic shoot; **D.** Abaxial blade; **E.** Infructescence; **F.** Spathe infructescenes splitting acroscopically and fruits were dispersed by unidentified red ant; **G**. Seeds.

sheath, 9 – 14 cm long x ca 8 mm wide, sheathing for ca $\frac{1}{3}$ of petiole length, persistent, membranous, fully attached with a short ligule, equal at both sides, slightly inrolled or sometimes straight, tapering, green with scattered dotting greenish striate; **blades** mostly ovato-sagittate, 28 – 35 cm long x 12 – 22 cm wide, softly coriaceous, adaxially glossy green, abaxially paler, posterior lobes subtriangular for 6 – 9 cm, sinus 8 – 9 cm across, apex acuminate for 2 – 2.5 cm, ultimately mucronate for ca 10 mm; **midrib** adaxially flush with blade, raised abaxially, ca 5 mm at insertion; **primary lateral veins** ca 14 per side, diverging at 30° – 80° from the midrib, raised adaxially towards the midrib, marginally impressed, entirely raised abaxially; **interprimary veins** adaxially weakly raised, alternating irregularly with primaries; few (0 – 2) **secondary veins** raised from each primary vein (1 – 2 secondary veins raised from primary veins near to insertion); **tertiary veins** inconspicuous; **vein-like pellucid glands** slightly visible. **Inflorescences** up to three, erect, emit esteric acid-like during pistillate anthesis, absent during staminate anthesis; **peduncle** 2 – 2.5 cm long x ca 5 mm wide, terete, green, erect at anthesis; **spathe** ca 11.5 cm long; **lower spathe** narrowly ovoid, ca 3.5 cm long x ca 1.6 cm wide, green, separated from spathe limb by a constriction coinciding with interstice; **spathe limb** turbinate, ca 6 cm long x ca 2.3 cm wide, mucronate for ca 3 mm, pale yellow at pistillate anthesis, slightly pallid prior to staminate anthesis, falls fresh in a single piece at onset of staminate anthesis; **spadix** ca 9 cm long, shorter than spathe, sessile; **pistillate flower zone** sub-cylindric**,** ca 4 cm long × ca 7 mm wide, ca $\frac{1}{2}$ length of spadix, light yellow; **pistils** sub-cylindric to sub-globose, ca 1 mm long x ca 0.5 mm wide, densely arranged at base, slightly laxly and impressed at apex of pistillate flower zone; **style** barely differentiated; **stigma** globose from above, truncated, equal with ovary, ca 0.5 mm diam., wet with stigmatic secretion at the onset of pistillate anthesis; **interpistillar staminodes** clavate, stipe slender, 92 – 130, ca 0.6 mm in diam., slightly exceeding pistils, white;

interstice cylindric, ca 3 mm long x ca 6 mm wide, weakly slender than pistillate and staminate flower zone, partially naked, comprised 2 – 5 whorls of clavate staminodes that resemble interpistillar staminodes, slightly laxly packed, staminodes and pistillodes not impressed; **staminate flower zone** cylindric, narrower at proximal but wider at distal, ca 2.5 cm long × 6 – 8 mm wide, ca $\frac{1}{3}$ length of spadix, yellowish white; **staminate flowers** ca 1 mm long x ca 0.5 mm wide, densely massed with individual flowers not discernible, bow tie shaped from above, each comprising 2 truncate stamens, sunken in, overtopping by a narrow connective; **pollen** powdery, white; **appendix** slender conoid, ca 2.5 cm long x ca 0.9 cm wide, ca $\frac{1}{3}$ length of spadix, base wider (ca 0.5 mm) than apex of staminate flower zone; **staminodes** sub-columnar, sub-globose towards the tip of appendix, ca 0.5 mm diam, densely arranged. **Infructescences** 1 – 3, 4.9 – 5.5 cm long x 1.4 – 1.6 cm wide, declinate; **lower spathe** entirely persistent, reflex when ripe; **fruits** ca 2 mm long x 1.5 – 2.5 mm wide, green to yellow; **seeds** ovoid ellipsoid, ca 0.4 mm diam., 7 – 10 per fruit, encased with greenish yellow gel.

Distribution – *Schismatoglottis pseudoniahensis* is known from its type locality, Niah National Park, Miri Division, Sarawak.

Ecology – Perhumid lowland tropical forests, adjacent to limestones, 13 m asl.

Etymology – The specific epithet is coined from the superficial similarity of this species to *S. niahensis* – hence *pseudo* – false.

Notes – There are several species in Niah National Park which belongs to the Calyptrata Group but with two species which looks very similar, *S. niahensis* A.Hay & *S. pseudoniahensis*. However, *S. niahensis* differs by having pleionanthic shoot and grows on the limestone outcrops where else *S. pseudoniahensis* has hapaxanthic shoot and grows along the muddy trails towards the Niah Caves.

Other specimens seen – Malaysia, Sarawak, Miri, Niah Suai, Niah N.P., Trail to Great Cave, 03.40 N, 113.47 E, 36 m asl, 26 December 2014, *S.Y.Wong & P.C.Boyce Ar5041* (SAR).

CHAPTER 4

POLLINATION BIOLOGY

4.1 Introduction

The pollination investigations in tribe Schismatoglottideae Nakai are scattering (Hay & Yuzammi, 2000). Several publications recorded that *Colocasiomyia* de Meijere flies play an importance role as the pollinator in *Schismatoglottis* Zollinger & Moritzi (*Schismatoglottis* spp., *S. calyptrata* (Roxb.) Zoll. & Moritzi, *S. wongii* A.Hay and *S. corneri* A.Hay) (Sultana *et al.,* 2006; Toda & Hakim, 2011) and *Ooia* S.Y.Wong & P.C.Boyce (*O. kinabaluensis* (Bogner) S.Y.Wong & P.C.Boyce and *O. grabowskii* (Engl.) S.Y.Wong & P.C.Boyce (previously known as *Piptospatha kinabaluensis* and *P. grabowskii*) (Wong & Boyce, 2010c; Toda & Hakim, 2011). However, these studies lack detailed field investigations. Recent detailed field investigations marked *Colocasiomyia* flies as the main pollinator in *Schottarum sarikeense* P.C.Boyce & S.Y.Wong (Low *et al.,* 2013; Low *et al.,* 2015), *Aridarum nicolsonii* Bogner (Low *et al.,* 2015), *Phymatarum borneense* M. Hotta (Low *et al.,* 2015) and *Bucephalandra* spp. (Wong & Boyce, 2014); *Chaloenus* Orchymont beetle as pollinator in one *Bucephalandra* sp. (Wong & Boyce, 2014); chrysomelid beetles (*Chaloenus* spp. and *Altica cyanae*) and *Cycreon* Orchymont beetles as insect visitors in *S. sarikeense* (Low *et al.,* 2013; Low *et al.,* 2015), *A. nicolsonii* (Low *et al.,* 2015) and *P. borneense* (Low *et al.,* 2015). In this chapter, the pollination biology of ten species of the *Schismatoglottis* Calyptrata complex was investigated in the aspects of: (1) field pollination (flowering mechanisms and insect activities), (2) insect visitations, (3) pollen count, (4) pollen view, (5) fruit set, (6) breeding test, (7) thermogenesis, and (8) floral scent analyses.

4.2 Materials and Methodology

4.2.1 Field Pollination Investigations- Flowering Mechanisms and Insect Activities

Inflorescences were identified and observed for a few days prior to the onset of anthesis. Flowering mechanisms (spathe opening, trapping and abscising, scent emission and pollen release) and insect activities (arrival, departure, eating (stigmatic secretion, interpistillar staminodes, staminate and appendix tissues, spathe, pollen), mating, fighting and egg depositing) were fully observed on *S. adducta* (n = 4) (15^{th} – 17^{th} May 2014), *S. baangongensis* (n = 5) (26^{th} – 28^{th} Oct 2011), *S. caesia* (n = 4) (5^{th} – 12^{th} Jan 2014), *S. calyptrata* (n = 4) (3^{rd} – 14^{th} Oct 2013), *S. giamensis* (n = 4) (2^{nd} – 5^{th} Aug 2012), *S. laxipistillata* (n = 4) (10^{th} Dec 2013 – 3^{rd} Jan 2014; 13^{th} – 18^{th} Jan 2014), *S. muluensis* (n = 5) (3^{rd} – 8^{th} Dec 2011), *S. pantiensis* (n = 4) (20^{th} Nov 2013 – 3^{rd} Dec 2013; 20^{th} – 26^{th} Jan 2014), *S. pseudoniahensis* (n = 4) (22^{nd} – 26^{th} Mar 2014), *S. roh* Ar1240 (n = 4) (6^{th} – 15^{th} Apr 2014) and *S. roh* Ar2445 (n = 4) (6^{th} – 15^{th} Apr 2014). Pollination was fully investigated during pistillate (0400 – 1300, day 1) and staminate anthesis (0400 – 1000, day 2) and partially investigated during inter-anthesis. All images were taken using a Panasonic Lumix DMC-LS85 or Nikon D3000 digital camera. The study locality, ecology, global position system (GPS) coordinate, voucher number and above sea level of the ten species of the Calyptrata complex were listed in **Table 4.1**.

Table 4.1. The study locality, ecology, global position system (GPS) coordinate, voucher number and above sea level of the ten species of the Calyptrata complex.

Taxon	Study locality	Ecology	GPS	Voucher number	Above sea level (m)
Schismatoglottis calyptrata	Ambon, Maluku, Indonesia.	Ca 200 plants occur on sandstone interbedded with coral limestone and ambon volcanic rock (andesite, dacite and tuff) (Menzie, *et al.,* 1997).	01.44 N; 113.28 E	Ar4270	46
Schismatoglottis roh Ar1240	Wind Cave, Krokong, Bau, Kuching Division, Sarawak, Malaysia.	100 – 150 plants occur 5 – 10 m away from the limestone cliff of the Wind Cave until the alluvium river margin.	01.24 N; 110.08 E	Ar1240	45
Schismatoglottis roh Ar2445	Fairy cave, Krokong, Bau, Kuching Division, Sarawak, Malaysia.	Ca 150 plants occur 5 – 10 m away from the limestone cliff of the Fairy Cave.	01.22 N; 110.07 E	Ar2445	30
Schismatoglottis baangongensis	Kampung Sikog, Siburan, Padawan, Kuching Division, Sarawak, Malaysia.	50 – 150 plants occur along the stream margin that is adjacent to karst limestone.	01.20 N; 110.20 E	Ar2588	70 – 75
Schismatoglottis giamensis	Kampung Giam, Siburan, Kuching Division, Sarawak, Malaysia.	100 – 150 plants occur along Giam waterfall stream margin with exposed limestone shale rocks (ca 150 ramets) or on a small cocoa plantation adjacent to karst limestone (ca 100 ramets).	01.19 N; 110.16 E	Ar2549	70
Schismatoglottis muluensis	Mulu National Park, Long Lama, Marudi, Miri Division, Sarawak, Malaysia.	Ca 300 plants occur on limestone karst along the Deer Cave trail.	04.02 N; 114.49 E	Ar1941	40
Schismatoglottis pseudoniahensis	Niah National Park, Niah Suai, Miri Division,	Ca 80 plants occur along the river margin (ca 80 ramets) or	03.49 N; 113.45 E	Ar4666	13

	Sarawak, Malaysia.	adjacent to limestone outcrop.			
Schismatoglottis adducta	Tempat Rekreasi Sungai Raya, Engkilili, Lubok Antu, Sri Aman Division, Sarawak, Malaysia.	100 – 250 plants occur on sandstone, along the alluvium waterfall stream margin.	01.06 N; 111.30 E	Ar1632	13
Schismatoglottis caesia	Kuala Koh National Park, Gua Musang, Kelantan, Peninsular Malaysia, Malaysia.	Ca 150 plants occur on sandstone, along the road margin until steep slope alluvium waterfall stream margin (ca 150 ramets).	04.52 N; 102.26 E	Ar4332	96
Schismatoglottis pantiensis	Hutan Simpan Panti, Kota Tinggi Division, Johor Bahru, Peninsular Malaysia, Malaysia.	Ca 120 plants occur along the waterfall stream margin.	01.48 N; 103.51 E	Ar4322	14
Schismatoglottis laxipistillata	Hutan Lipur Rekreasi Tupah, Bedong, Merbok, Kedah, Peninsular Malaysia, Malaysia.	Ca 30 plants restricted on a steep granite slopes (150 m^2 area) that inundated with sandstone mud, along the waterfall stream margin.	05.44 N; 100.26 E	Ar4331	91

4.2.2 Insect Visitations

An independent set of inflorescences were further bagged during pistillate anthesis for mean ± standard deviation (SD) of number of insects: *S. adducta* (n = 7)*, S. baangongensis* (n = 8), *S. caesia* (n = 3), *S. calyptrata* (n = 4), *S. giamensis* (n = 9), *S. laxipistillata* (n = 4), *S. muluensis* (n = 6), *S. pantiensis* (n = 3), *S. pseudoniahensis* (n = 5), *S. roh* Ar1240 (n = 4) and *S. roh* Ar2445 (n = 7). The visitation number among the insect groups in each species of Calyprata complex was tested with Friedman test. Wilcoxon test was used for pairwise comparisons. Insects were identified to the lowest possible taxonomic level (at least family level) by Masanori J. Toda (*Colocasiomyia* flies and Chironomidae flies), Haruo Takizawa (*Chaloenus*

beetles), Kaoru Wada (*Parastasia* beetle), Masahito T. Kimura (Pteromalidae wasps and *Trigona* bees), Maruyama Munetoshi (*Atheta* beetles), Alexander G. Kirejtshuk (*Cycreon* beetles) and Martin Fikáček (*Cycreon* beetles). Insect specimens were preserved in 70% ethanol and deposited in Sarawak Zoology museum.

4.2.3 Fruit Set

To measure the effectiveness of pollination, natural fruit set was defined as the ratio between total fruits per infructescence and total pistillate flowers per inflorescence: *S. adducta* (n = 7 infructescences/5 inflorescences), *S. baangongensis* (n = 7/6), *S. caesia* (n = 5/6), *S. calyptrata* (n = 6/5), *S. giamensis* (n = 7/12), *S. laxipistillata* (n = 5/6), *S. muluensis* (n = 5/5), *S. pantiensis* (n = 6/5), *S. pseudoniahensis* (n = 5/5), *S. roh* Ar1240 (n = 9/5) and *S. roh* Ar2445 (n = 12/9). Seed set was estimated by counting the number of developed seeds on 10 fruits from 5 infructescences per species (n = 50).

To further confirm the effective pollination by smallest insects (*Colocasiomyia* fly, *Cycreon* beetle and *Atheta* Thomson beetle), fruit set was counted on bagged inflorescences of *S. baangongensis* (n = 4), *S. giamensis* (n = 5) and *S. roh* Ar2445 (n = 7) with a 2 mm x 2 mm mesh net, allowing access only to smallest insects. The mesh net was removed at the end of the anthesis period. Mann-Whitney pairwise comparisons tests was applied for testing the differences between the natural fruit set and bagged fruit in *S. baangongensis*, *S. giamensis* and *S. roh* Ar2445.

To test the breeding system of the plants, fruit set for spontaneous self-pollination was measured by covering the inflorescences of *S. baangongensis* (n = 4), *S. giamensis* (n = 5) and

S. roh Ar2445 (n = 4) with organdy bags during all the anthesis that prevent the visitation of the insects. Mann-Whitney pairwise comparisons tests was applied for testing the differences between the natural fruit set and bagged fruit in *S. baangongensis*, *S. giamensis* and *S. roh* Ar2445.

4.2.4 Pollen Count

Based on the availability of the inflorescence specimens, the pollen count only investigated for *S. baangongensis, S. giamensis* and *S. roh* Ar1240. To analyse the mean ± SD of number of adhered pollen on the visited insects, insects were sampled at the onset of pistillate anthesis (0600 – 0900) from four inflorescences of *S. baangongensis* (*Colocasiomyia* flies (n = 12), *Cycreon* (n = 13), *Chaloenus* (n = 2) and *Atheta* beetle (n = 1)); three inflorescences of *S. roh* Ar1240 (*Colocasiomyia* flies (n = 14), *Cycreon* beetles (n = 5), *Chaloenus* beetles (n = 3)) and three inflorescences of *S. giamensis* (*Colocasiomyia* flies (n = 12), *Cycreon* beetles (n = 8), *Chaloenus* beetles (n = 10) and *Atheta* beetle (n = 1)). A polyethylene bag with perforations (ca 1 mm) was used to cover the inflorescence prior to arrival of the insects. At the onset of pistillate anthesis, insects that landed on the covered inflorescence was collected into 2 ml collecting tubes and preserved in -20 °C until the analysis. During counting, individual insect was washed with 200 ul 70 % ethanol, 50 uL of the wash ethanol was transferred to the Sedgewick Rafter Counting Chamber (S52-glass) and remained covered until completely evaporated (slightly modified from Pereira *et al.,* 2014). The counted pollen grain for each individual insect was multiplied by four. A compound microscope Olympus BX51 which is equipped with Olympus DP72 camera and Cell ^D software, version 3.3 (Build 2067) were used. During counting, the pollen was identified by observing the host pollen (collected at the onset of staminate anthesis and preserved in -20 °C until the analysis) under the microscope.

Kruskal-Wallis tests and Mann-Whitney pairwise comparisons tests were applied for testing the differences of adhered pollen among the insect groups.

4.2.5 Pollen View

Base on the availability of the insect specimens, the pollen view only investigated for *S. baangongensis* and *S. giamensis*. To further confirm the adhered pollen on *Colocasiomyia* flies and *Cycreon* beetles, both insects were collected (similar in section 4.2.4) and were dried under the silica gel for three days, coated with gold using JOE JFC-1600 auto-fine coater and images were captured using JEOL JSM-6390LA analytical scanning electron microscope (SEM). To identify the host pollen, pollen collecting (4.2.4), drying and coating (4.2.5) were repeated.

4.2.6 Breeding Test

Owing to the fact that inflorescence parts were infected by fungi, the larval growth success of visiting insects on different inflorescence parts were only investigated on pistillate flower zone (n = 2) of *S. giamensis,* spathe limb (n = 1) and pistillate flower zone (n = 1) of *S. roh* Ar2445. Different inflorescence parts were collected (0800 – 1200, staminate anthesis), placed on each different Petri dishes, kept under room temperature (24°C – 32°C) and 250 ul of the distilled water was added daily to keep the condition moist. The number of hatched insects was observed and counted each day for a month.

4.2.7 Thermogenesis

Due to no source of electricity in the field or no flowering buds, the floral temperature patterns of *S. calyptrata* (four inflorescences from four individual plants), *S. adducta* (three

inflorescences during pistillate anthesis, two inflorescences during staminate anthesis from two individual plants), *S. giamensis* (two inflorescences at both pistillate and staminate anthesis from two individual plants), *S. pseudoniahensis* (four inflorescences at both pistillate and staminate anthesis from two individual plants) and *S. roh* Ar1240 (two inflorescences at both pistillate and staminate anthesis from two individual plants) were only investigated.

The temperature of the spadix zones (appendix, staminate, pistillate flower zone) and ambient were recorded *in-situ* (*S. giamensis* and *S. roh* Ar1240) and plants cultivated in the research collection (double layer of 50% shade netting structures surrounded by natural forests) (*S. adducta*, *S. calyptrata* and *S. pseudoniahensis*) for the whole anthesis at 5 minute intervals. A personal laptop (supplied with electricity via a 20 m extension cable) connected to a USB TC-08 Thermocouple Data Logger (Pico Technology) with four probes (Type K) was used: three probes were inserted ca 0.5 cm into the middle part of the appendix, staminate and pistillate flower zone respectively, another probe was hung freely at the same height for ambient temperature.

4.2.8 Floral Volatile Organic Compounds (VOCs) Analyses

Sampling of floral scent was *in situ* collected for nine investigated species belonging to the Calyptrata complex (*S. baangongensis* (n = 6), *S. calyptrata* (n = 6), *S. roh* Ar2445 (n = 6), *S. giamensis* (n = 5), *S. caesia* (n = 6), *S. muluensis* (n = 6), *S. pantiensis* (n = 4), *S. adducta* (n = 6), *S. laxipistillata* (n = 6), *S. roh* Ar1240 (n = 4), *S. pseudoniahensis* not sampled due to not enough of inflorecences in the field) using the dynamic headspace method. Floral scent of inflorescences was trapped for two hours between 0530 – 0800, for pistillate (all Calyptrata species) and staminate anthesis (*S. baangongensis*, *S. calyptrata* and *S. muluensis*). Owing to

floral scent was not detected at staminate anthesis (Gas Chromatography-Mass Spectrometry analyses in *S. baangongensis*, *S. calyptrata* and *S. muluensis* and field investigations of all Calyptrata species), staminate anthesis of other species were not trapped. During onset of the pistillate anthesis, a separate set of spadix inflorescences were dissected and each different inflorescence part was trapped twice: 0600 – 0800 (period I) [*S. baangongensis*: appendix (n = 2), pistillate flower zone (n = 4), spathe (n = 3) and staminate flower zone (n = 3), *S. giamensis*: appendix (n = 3), pistillate flower zone (n = 3), spathe (n = 4) and staminate flower zone (n = 2) and *S. adducta*: appendix (n = 3), pistillate flower zone (n = 3), spathe (n = 3) and staminate flower zone (n = 4)] and 0815 – 1015 (period II) [(*S. baangongensis*: appendix (n = 2), pistillate flower zone (n = 2), spathe (n = 2) and staminate flower zone (n = 4), *S. giamensis*: appendix (n = 3), pistillate flower zone (n = 2), spathe (n = 2) and staminate flower zone (n = 2) and *S. adducta*: appendix (n = 3), pistillate flower zone (n = 2), spathe (n = 3) and staminate flower zone (n = 2)]. Inflorescences or different inflorescence parts were individually enclosed within PET film oven bags (EasyRoast™, Bacofoil, UK), the floral volatile organic compounds (VOCs) in the air were drawn by battery-operated vaccum pumps (Spectrex PAS-500 Micro Air Sampler; Spectrex, USA) and were trapped in glass tubes containing adsorbent polymer (ORBO™ 402 Tenax® TA 35/60 mesh, 100/50 mg; Sigma-Aldrich, USA) at a constant flow rate of 200 $ml.min^{-1}$. Control samples were simultaneously collected using empty oven bags. When sampling was not carried out, inflorescences were covered with fine organdy bags to prevent insect visitation. The adsorbent traps were eluted with hexane (4 mL; ≥ 98.5% purity, MERCK, Germany) and subsequently kept at -20 °C refrigeration until further analysis.

4.2.8.1 Chemical Analysis of Floral VOCs

All samples were analyzed on a SHIMADZU GC-2010 and a SHIMADZU GC-MS-QP2010 Plus. A BP-5 intermediate polar column (29.5 m long, 0.23 mm inner diameter and film thickness 0.25 μm) was used to analyze the inflorescences, for pistillate anthesis [*S. roh* Ar1240 (n = 1), *S. roh* Ar2445 (n = 2), *S. giamensis* (n = 3), *S. baangongensis* (n = 6), *S. adducta* (n = 3), *S. muluensis* (n = 2) and *S. calyptrata* (n = 5)] and staminate anthesis [*S. baangongensis* (n = 1), *S. calyptrata* (n = 2) and *S. muluensis* (n = 2)]. Prior to the analyses, 20 μL of tetradecane (internal standard, 13 ng/μL) was mixed with 700 μL eluted sample and concentrated to ca 80 μL under the fumehood. 1 μL of each concentrated sample was injected in splitless mode, GC oven temperature was set at 35 °C for 5 min, increased at a rate of 5 °C min^{-1} to 180 °C, then 10 °C min^{-1} to 200 °C and held steady for 10 min (Hoe, 2012).

The second BP20 polar column (30 m long, 0.25 mm inner diameter and film thickness 0.25 um) was used to analyze the inflorescences, for pistillate anthesis [*S. baangongensis* (n = 6), *S. calyptrata* (n = 6), *S. roh* Ar2445 (n = 6), *S. giamensis* (n = 5), *S. caesia* (n = 6), *S. muluensis* (n = 6), *S. pantiensis* (n = 4), *S. adducta* (n = 6), *S. laxipistillata* (n = 6), *S. roh* Ar1240 (n = 4)], different inflorescence parts for period I [*S. baangongensis*: appendix (n = 2), pistillate flower zone (n = 4), spathe (n = 3) and staminate flower zone (n = 3), *S. giamensis*: appendix (n = 3), pistillate flower zone (n = 3), spathe (n = 4) and staminate flower zone (n = 2) and *S. adducta*: appendix (n = 3), pistillate flower zone (n = 3), spathe (n = 3) and staminate flower zone (n = 4)] and period II [(*S. baangongensis*: appendix (n = 2), pistillate flower zone (n = 2), spathe (n = 2) and staminate flower zone (n = 4), *S. giamensis*: appendix (n = 3), pistillate flower zone (n = 2), spathe (n = 2) and staminate flower zone (n = 2) and *S. adducta*: appendix (n = 3), pistillate flower zone (n = 2), spathe (n = 3) and staminate flower zone (n =

2)]. 5 µL of tetradecane (internal standard, 13 ng/µL) was mixed with 600 µL eluted sample and concentrated to ca 80 µL under the fumehood. 1 µL of each concentrated sample was injected in splitless mode, but the major compound saturated the column, thus injections was changed to split mode (1:20). The GC oven temperature was set at 50 °C for 5 min, increased at a rate of 5 °C min^{-1} to 260 °C and held steady for 10 min. Both columns were set to 200 °C for the injector temperature, carrier gas flow was maintained at a constant pressure of 100 psi, MS Source and quadrupole temperatures were set at 220 °C and 200 °C respectively and mass spectra were taken from *m/z* 35 – 500 in EI (electron ionization) mode. The Kovats Retention Indexes (KIs) of the VOCs were obtained with an external standard (C_7 – C_{22} saturated alkanes, Supelco, USA). Compounds were identified by comparing their mass spectra and retention times with those of authentic reference samples available from commercially available mass spectral libraries (nist08 Library Mass Spectral Database). The peak areas on the chromatograms were integrated to determine the relative amounts of each compound. The chromatograms were listed in **Appendix 1 – 5**.

4.2.8.2 Statistical Analyses of the Floral VOCs

All statistical analyses on floral VOCs were performed using Paleontological Statistics (PAST) version 2.17 (Hammer *et al.,* 2001). Mann-Whitney test was performed for the total amount of different inflorescence parts (appendix, spathe, pistillate flower zone and staminate flower zone) between time periods I and II. One-way PERMANOVA (9999 permutations, Bray-Curtis distance index) was applied to test the differences in floral scent profiles among species inflorescence, with sequential Bonferroni significance for pairwise comparisons. The floral VOCs was further tested in two-way PERMANOVA (9999 permutations, Bray-Curtis distance index) with the fixed factors (i. locality, ii. pollinator: present of *Colocasioymia* fly

and *Cycreon* beetle, iii. Opportunist pollinator: present of *Parastasia* beetle) in a two-way crossed design (Gottsberger *et al.,* 2013). A non-metric multidimensional scaling (NMDS) was used to depict variation in floral scent among the taxa (Steiner *et al*., 2011) using Bray-Curtis distance index (Bray & Curtis 1957) with zero environmental variables. The stress values below 0.1 represent a good ordination in the interpretation.

4.3 Results

4.3.1 Flowering Mechanisms, Pollination Strategies, and Insect Activities

4.3.1.1 *Schismatoglottis calyptrata*

The total anthetic period of *S. calyptrata* was ca 26 ¼ hours: pistillate anthesis (wet pistils) started at 0430 (local time) and the staminate anthesis (pollen release) on the following day, at 0530 (**Figure 4.1, 4.2**). At the onset of pistillate anthesis, the spathe loosened and a gap was formed along the spathe limb and lower spathe margin. A mild esteric scent was emitted from the inflorescence, turned intensified (0600), later reduced (1000) and ended (1430). By 0630, the spathe limb's colour turned from green to pale yellowish green, opened wide (ca 8.4 cm long x ca 2.8 cm wide), revealed a spathe limb opening (ca 1.8 cm long x ca 5 mm wide) and lower spathe inflated (ca 3.6 cm long x ca 1.6 cm wide). Between 0745 – 0845, a moderately constricting spathe trapping device was formed. It tightened its spathe limb (ca 2.7 cm wide) and lower spathe (ca 1.4 cm wide), spathe limb opening was reduced (ca 1.0 cm long x ca 2 mm wide) or occasionally fully closed (however, insects still could escape from the marginal of the spathe limb). At the onset of staminate anthesis on the following day, the spathe limb started to abscise (0520) and the lower staminate flower zone began to release the pollen

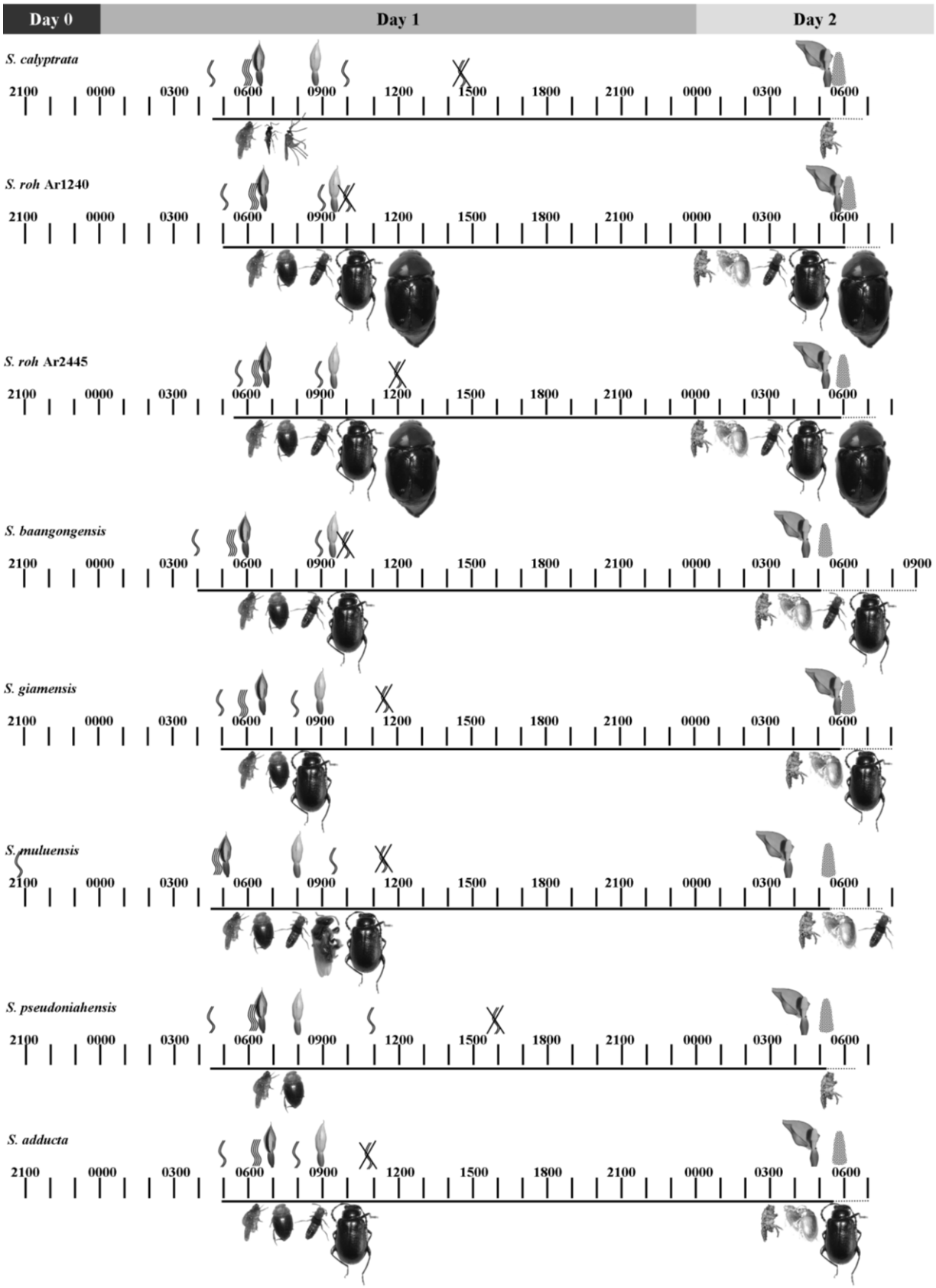

Figure 4.1. Flowering mechanisms and insect activities for ten investigated species of the Calyptrata complex.

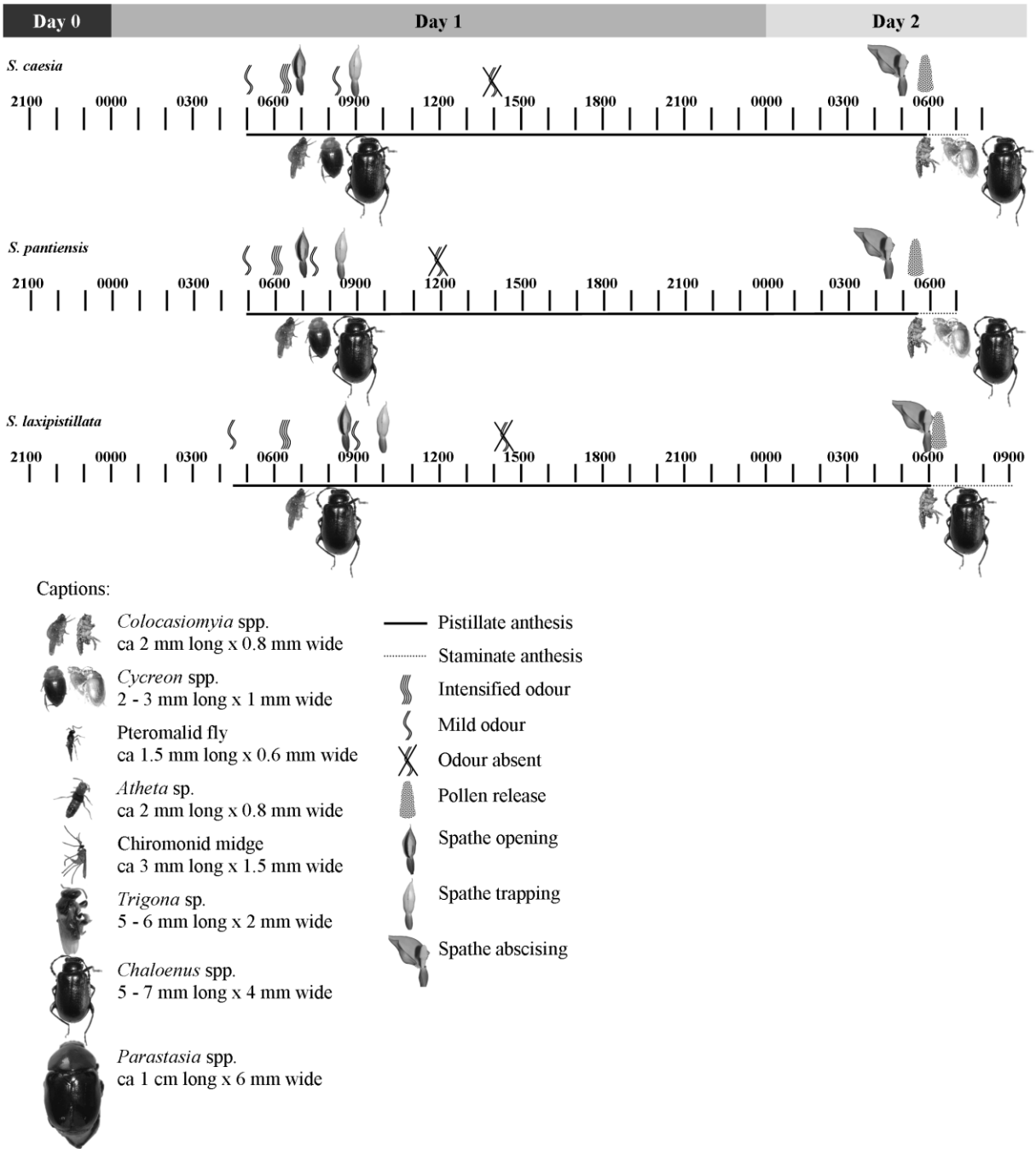

Figure 4.1. Flowering mechanisms and insect activities for ten investigated species of the Calyptrata complex.

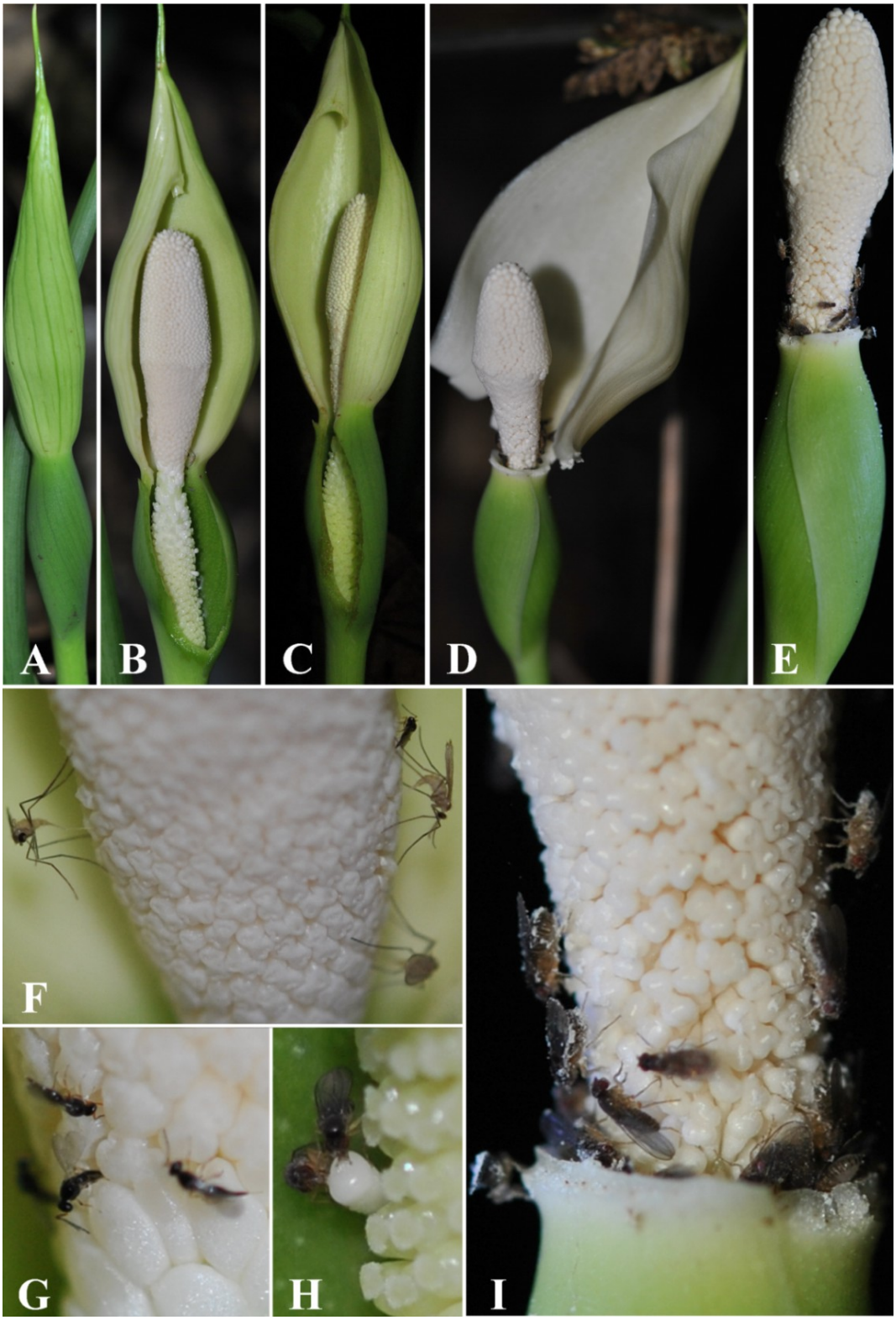

Figure 4.2. Flowering mechanisms and insect activities of *S. calyptrata*. **A – E.** Flowering sequence: (A) a day prior to anthesis; (B) pistillate anthesis with spathe was artificially being removed; (C) spathe trapping; (D) spathe abscising; (E) pollen release; **F – G.** Chironomid midges (F) and *Pteromalid* wasps (G) lay eggs on staminate flower zone; **H.** *Colocasiomyia* flies sucking the interpistillar staminodes; **I.** Pollen adhere on *Colocasiomyia* flies that consume the pollen.

(0530). The spathe limb was fully abscised (0535) and white powdery pollen was extended throughout the whole staminate flower zone (0545). The fruiting development was not observed.

The mean ± SD of caught insects per inflorescence were: *Colocasiomyia* flies (Diptera: Drosophilideae; 39 ± 15), Pteromalid wasps (Pteromalidae; 2 ± 2) and Chironomid midges (Chironomidae; 3 ± 3) (**Table 4.2**, **Figure 4.3**). The number of visiting individuals among the insect groups was significantly different (Friedman test, $p < 0.03$). Species of *Colocasiomyia* was identified as *C.* aff. *bogneri*. At the onset of pistillate anthesis, the floral scent release attracted firstly *Colocasiomyia* flies (0550), and between 0610 – 0615, their arrival was the most abundant. These flies were highly active, moved freely within the inflorescence, mainly engaged in courtship and mating activity on the lower staminate flower zone, and some mated on inner spathe limb and pistillate flower zone. *Colocasiomyia* flies sucked on the surface of the interpistillar staminodes, fed on the liquid secreted from the stigma and inner surface of the spathe limb. When the scent reduced (1000), they were inactive and remained inside the lower chamber until the following day. The Chironomid midges (0610) and Pteromalid wasps (0645) arrived, moving around the staminate flower zone and were seen to deposite eggs. They rarely visited the appendix, never reach pistillate flower zone and left after a short visitation (Chironomid midges, 0800; Pteromalid wasps, 1200). At the onset of staminate anthesis, *Colocasiomyia* flies shifted to lower staminate flower zone to consume the pollen and pollen was adhered on their body. Prior to leaving, *Colocasiomyia* flies clean themselves by flipping their wings and wiping their head parts and abdomen by using their fore and hind legs. However, powdery pollen still remained adhered and by dawn they moved to the tip of appendix and left (0555 – 0645). This marked the end of the anthesis of *S. calyptrata*.

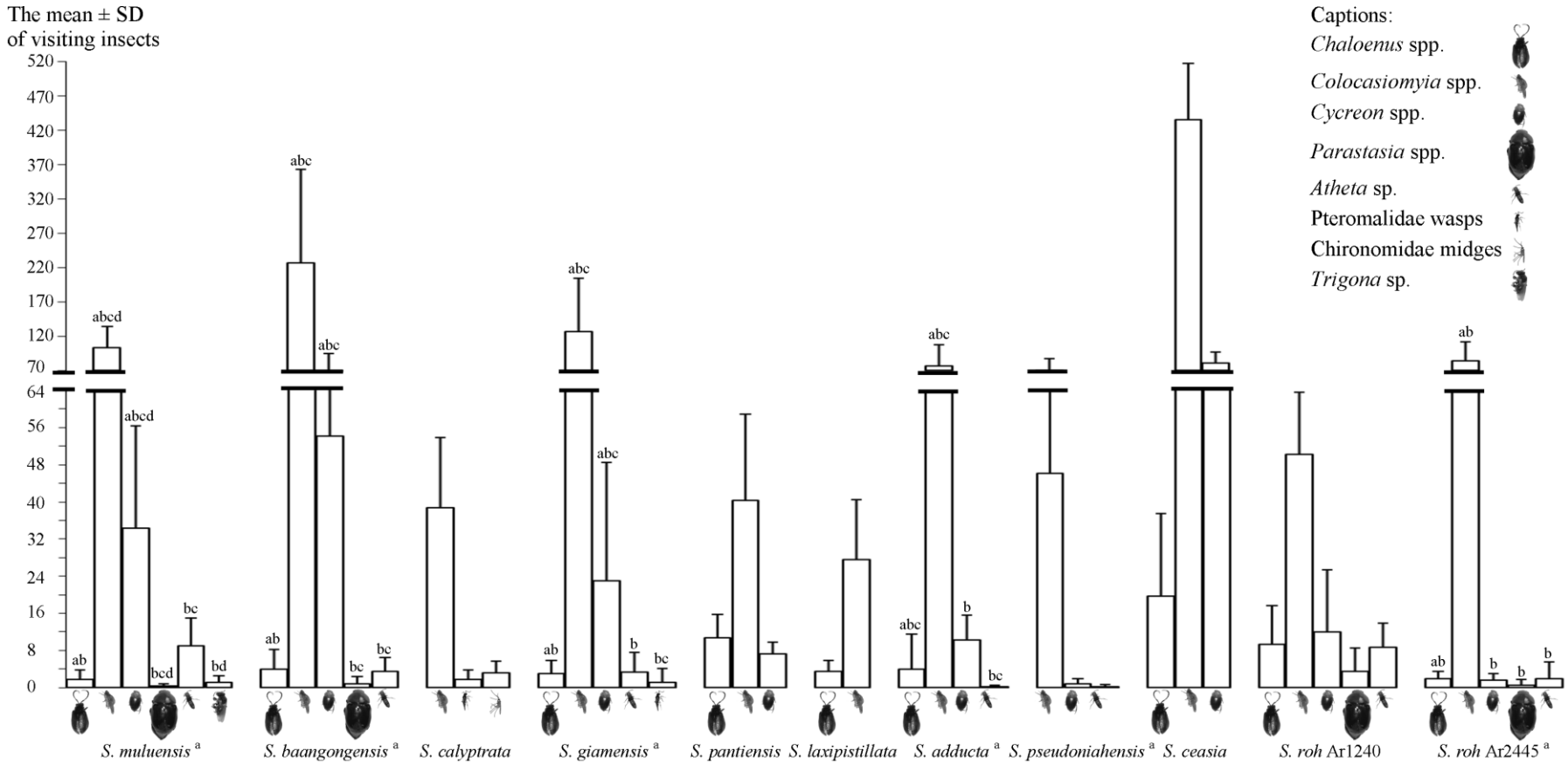

Figure 4.3. The mean ± standard deviation of number of visiting insects per inflorescence for the investigated species of the Calyptrata complex. Significant different (Wilcoxon pairwise comparisons tests) for the visitation number between insect groups are indicated with different alphabets.

Table 4.2. The mean ± standard deviation of number of visiting insects per inflorescence for the investigated species of the Calyptrata complex. Significant different (Wilcoxon tests) for the visitation number between insect groups are indicated with different alphabets.

Plant taxa	Flower visitor taxa							
	Chaloenus spp.	*Colocasiomyia* spp.	*Cycreon* spp.	*Parastasia* spp.	*Atheta* sp.	*Trigona* sp.	Pteromalidae wasps	Chironomidae midges
S. baangongensis (n = 9)	*C. dohertyi* + *C. latifrons* + *C. schawalleri* + sp. 1 4 ± 4 [ab]	*C.* aff. *bogneri* 227 ± 137 [abc]	-- 54 ± 41 ab[abc]	*P. gestroi* + *P. nigripennis* 1 ± 2 [ac]	-- 3 ± 3 a[ac]	0	0	0
S. calyptrata (n = 4)	0	*C.* aff. *bogneri* 39 ± 15	0	0	0	0	-- 2 ± 2	-- 3 ± 3
S. roh Ar2445 (n = 7)	*C. latifrons* 2 ± 2 [ab]	*C.* aff. *bogneri* 84 ± 28 [ab]	-- 2 ± 2 [a]	*P. nigripennis* 1 ± 1 [a]	-- 2 ± 4 [a]	0	0	0
S. giamensis (n = 10)	*C. latifrons* + *C. schawalleri* + sp. 1 + sp. 3 3 ± 3 [ab]	*C.* aff. *bogneri* 127 ± 78 [abc]	-- 23 ± 25 [abc]	0	-- 3 ± 4 [a]	0	-- 1 ± 3 [ac]	0
S. caesia (n = 3)	*C. latifrons* 20 ± 18	-- 436 ± 82	-- 81 ± 16	0	0	0	0	0
S. muluensis (n = 6)	*C. dohertyi* + *C. schawalleri* 2 ± 2 [ac]	*C.* aff. *bogneri* 103 ± 31 [abcd]	-- 34 ± 22 [abcd]	*P. nigripennis* 1 ± 1 [acd]	-- 9 ± 6 [ac]	-- 1 ± 1 [ad]	0	0
S. pseudoniahensis (n = 5)	0	-- 46 ± 41	-- 1 ± 1	0	-- 1 ± 1	0	0	0
S. pantiensis (n = 3)	*C. latifrons* 11 ± 5	-- 40 ± 19	-- 7 ± 3	0	0	0	0	0
S. adducta (n = 7)	*C. schawalleri* + *C. latifrons* + sp. 3 4 ± 8 [abc]	*C.* aff. *bogneri* 77 ± 31 [abc]	-- 10 ± 5 [a]	0	-- 1 ± 1 [ac]	0	0	0
S. laxipistillata (n = 4)	*C. latifrons* 4 ± 2	-- 28 ± 13	0	0	0	0	0	0
S. roh Ar1240 (n = 4)	*C. latifrons* + *C. schwalleri* 9 ± 8	-- 50 ± 13	-- 12 ± 13	*P. nigripennis* 4 ± 5	-- 9 ± 5	0	0	0

Colocasiomyia flies are considered as the main pollinator of *S. calyptrata*. Their arrival and departure correlated with the spathe opening and scent release. During pistillate anthesis, *Colocasiomyia* flies sucked on the surface of the interpistillar staminodes and adhered pollen was transferred to the wet pistils. During staminate anthesis, *Colocasiomyia* flies consumed the pollen and left with adhered pollen. The Chironomid midges and Pteromalid wasps are considered as visitors that seeked for breeding site as they never visited the pistillate flower zone and were absent during pollen release.

4.3.1.2 *Schismatoglottis roh* Ar1240

The total anthetic period of *S. roh* Ar1240 was ca 26.5 hours: pistillate anthesis (wet pistils) started at 0500 and the staminate anthesis (pollen release) on the following day, at 0600 (**Figure 4.1, 4.4**). At the onset of pistillate anthesis, the lower spathe loosened to ca 3 mm marginal gap opening, yet the spathe limb remained tightened. A mild esteric scent was emitted (0500), turned intensified (0630), later reduced (0900) and ended (1000). By 0630, the spathe limb's colour turned from green to pale yellowish green, opened wide (ca 7.5 cm long x ca 2.5 cm wide) and lower spathe inflated (ca 3.8 cm long x ca 1.8 cm wide). From 0800 – 0930, a moderately constricting spathe trapping device was formed. It tightened its spathe limb (ca 3.2 cm wide) and lower spathe (ca 1.7 cm wide), spathe limb opening was fully enclosed (however, insects still could escape from the marginal of the spathe limb). At the onset of staminate anthesis on the following day, the spathe limb started to abscise (0550) and fully abscised by 0610. White powdery pollen began to be released on the lower staminate flower zone (0600), and extended throughout the whole staminate flower zone (0615). After 35 – 40 days, the fruiting spathe splitted and fruits were released from dawn onwards. The fruiting spathe senescence mechanism was not fully observed.

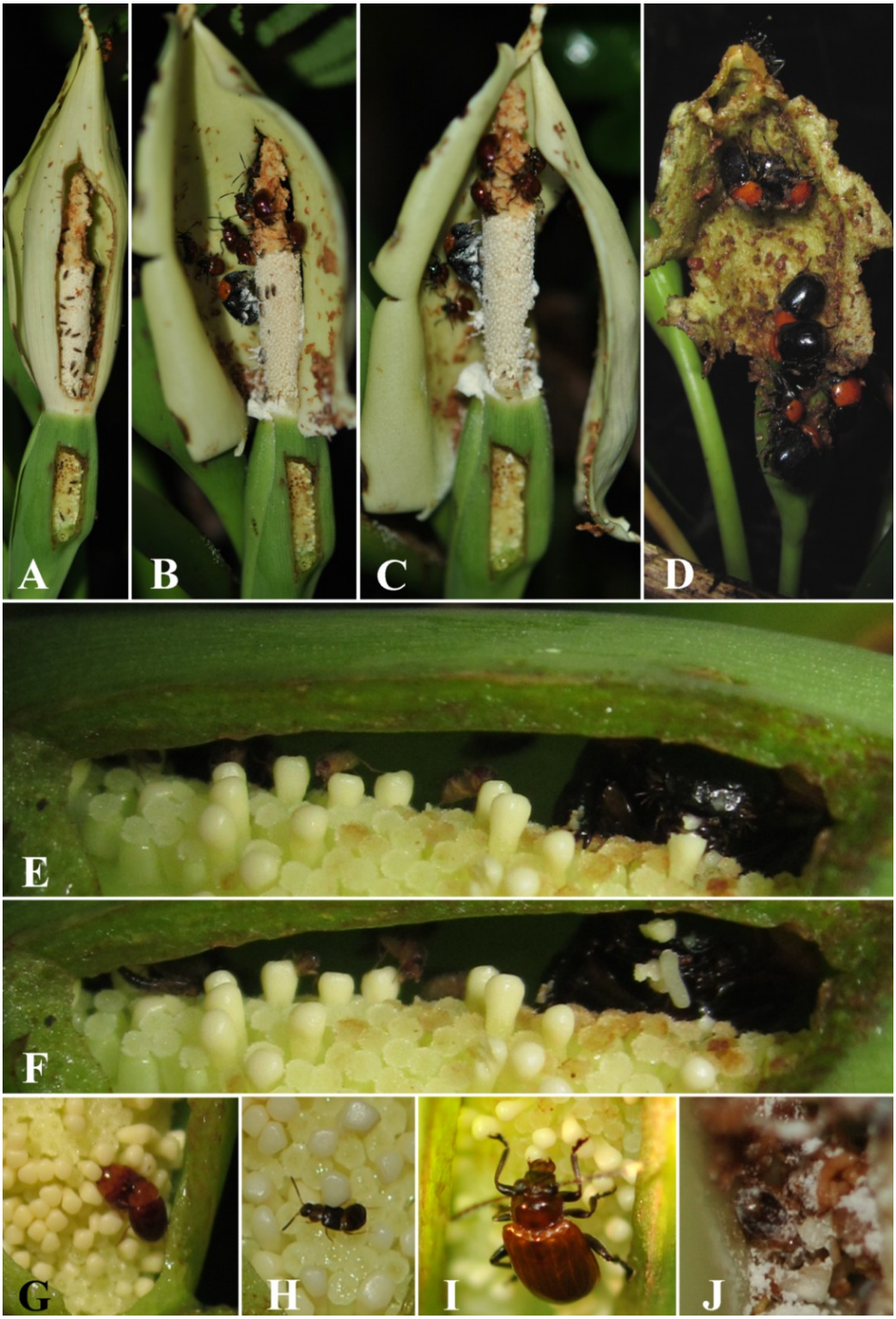

Figure 4.4. Flowering mechanisms and insect activities of *S. roh* Ar1240. **A**. Inflorescence fully flowering; **B – C**. Spathe abscising and pollen adhere on *Colocasiomyia* flies that consume the pollen. *Parastasia* beetle was forced to remain on staminate flower zone; **D.** *Parastasia* beetles damaging the small solitary inflorescence; **E – F.** *Parastasia* beetles chewing and dislodged the interpistillar staminodes; **G.** *Cycreon* beetles mating on pistillate flower zone; **H.** *Atheta* beetle is moving between the pistils; **I.** *Chaloenus* beetle chewing the interpistillar staminode; **J.** Pollen adhere on *Cycreon* beetle.

The mean ± SD of caught insects per inflorescence were: *Chaloenus* beetles (9 ± 8), *Colocasiomyia* flies (50 ± 13), *Cycreon* beetles (12 ± 13), *Atheta* beetles (Aleocharinae; 9 ± 5), and *Parastasia* beetles (4 ± 5) (**Table 4.2, Figure 4.3**). The number of visiting individuals among the insect groups was significantly different (Friedman test, $p < 0.03$). However, during the latest field trip in July 2014, *Parastasia* beetles were not found in the four partially observed inflorescences (pistillate anthesis, 0800 – 1200). Species of *Chaloenus* beetles were identified as *C. latifrons* and *C. schwalleri*; *Parastasia* beetles were identified as *P. nigripennis*.

At the onset of pistillate anthesis, the released floral scent attracted firstly *Colocasiomyia* flies (0630). *Colocasiomyia* flies were highly active, moved freely within the inflorescence, mated on inner spathe and spadix (appendix, staminate and pistillate flower zone). These flies sucked on the surface of the interpistillar staminodes and inner spathe limb and fed on the liquid secreted from the stigma. *Cycreon* beetles (0630) sucked the interpistillar staminodes. *Chaloenus* beetles arrived (0715), chewed the appendix and lower staminate flower zone, some fighting on appendix. Between 0715 – 0830, *Atheta* beetles arrived and moved among the pistils). *Parastasia nigripennis* arrived (0700) and squeezed itself through the middle spathe limb margin and moved to the pistillate flower zone to chew on the interpistillar staminodes. When the floral scent reduced (0900), all insects were less active. *Colocasiomyia* flies mostly remained on the inner spathe limb and inner lower spathe, some still sucking on the interpistillar staminodes. *Chaloenus* beetles mostly remained on the appendix, others were observed to be mating on the inner spathe limb. An individual of *Chaloenus* was observed to be chewing on the interpistillar staminodes. *Parastasia nigripennis* was still chewing on the

interpistillar staminodes. *Cycreon* beetles remained inside the lower chamber until the following day.

At the onset of staminate anthesis, the appendix was mostly gnawed by *P. nigripennis* and *Chaloenus* beetles. Both beetles remained on the appendix. However, in one observed inflorescence owing to the crowded space on appendix, two *Chaloenus* beetles were forced to shift to the inner spathe limb while an individual of *P. nigripennis* remained on the upper staminate flower zone (0500 – 0600). Pollen was seen to be fully adhered onto *P. nigripennis.* Up to 11 individuals of *P. nigripennis* were observed to severely damage the appendix and the stamens of an inflorescence prior to pollen release. *Colocasiomyia* flies consumed the pollen on the whole staminate flower zone while *Cycreon* beetles consumed the pollen on the lower staminate flower zone. Pollen was fully adhered on *Colocasiomyia* flies and *Cycreon* beetles. Prior to departure, some *Colocasiomyia* flies crawled onto *Chaloenus* beetles and *P. nigripennis* and removed the adhered pollen on both beetles. *Atheta* beetles were found on pistillate flower zone (0500) but left during pollen release. By 0730, all insects left the inflorescence and this marked the end of anthesis of *S. roh* Ar1240.

Colocasiomyia flies and *Cycreon* beetles are considered as the main pollinators of *S. roh* Ar1240. Their arrivals were correlated with the spathe opening and floral scent release. During pistillate anthesis they sucked on the surface of interpistillar staminodes and thus adhered pollen was transferred to the wet pistils. During staminate anthesis they consumed the pollen and left with adhered pollen. *Chaloenus* beetles and *P. nigripennis* are considered as the opportunistic pollinators as they were destructive (gnawed the spadix) and remained on the appendix during pollen release. Pollen adhered on *Chaloenus* beetles and *P. nigripennis*

due to *Colocasiomyia* flies crawling onto *Chaloenus* beetles and *P. nigripennis*. *Chaloenus* beetles and *P. nigripennis* remained on the upper staminate flower zone owing to the crowded space on appendix during pollen release. *Atheta* beetles are considered as visitor as they were not found to feed on the pollen.

4.3.1.3 *Schismatoglottis roh* Ar2445

The total anthesis of *S. roh* Ar2445 was ca 25 ¾ hours: pistillate anthesis (wet pistils) started at 0530 and the staminate anthesis (pollen release) on the following day, at 0600 (**Figure 4.1, 4.5**). At the onset of pistillate anthesis, a mild esteric scent was emitted (0530), turned intensified (0630), later reduced (0900) and ended (1200). By 0600, the spathe limb's colour turned from green to pale yellowish green, opened wide (0645) (ca 7.5 cm long x ca 3.0 cm wide) and lower spathe inflated (ca 4 cm long x ca 1.8 cm wide). Between 0800 – 0930, a moderately constricting spathe trapping device was formed. It tightened its spathe limb (ca 2.35 cm wide) and lower spathe (ca 1.6 cm wide), spathe limb opening was fully enclosed (however, insects still could escape from the chamber). At the onset of staminate anthesis on the following day, the spathe limb started to abscise (0510) and fully abscised by 0540. White powdery pollen began to be released on the lower staminate flower zone (0600), and extended throughout the whole staminate flower zone (0635). After 35 – 40 days, the fruiting spathe splitted and fruits were released from dawn onwards. The fruiting spathe senescence mechanism is not fully observed.

The mean ± SD of caught insects per inflorescence were *Chaloenus* beetles (2 ± 2), *Colocasiomyia* flies (84 ± 28), *Cycreon* beetles (2 ± 2), *Parastasia* beetles (1 ± 1), *Atheta* beetles (2 ± 4) (**Table 4.2, Figure 4.3**). The number of visiting individuals among the insect

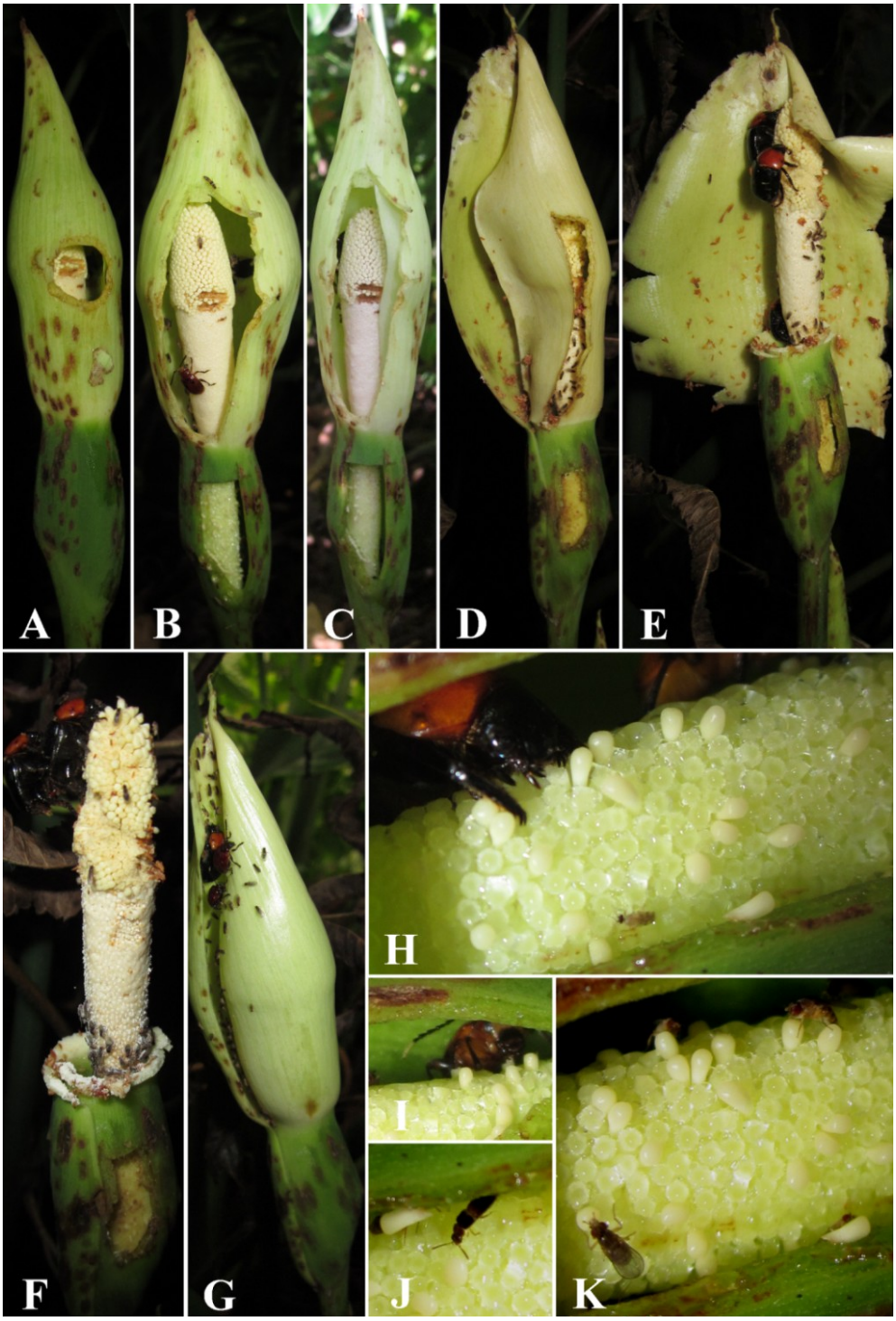

Figure 4.5. Flowering mechanisms and insect activities of *S. roh* Ar2445. **A – F.** Flowering sequence: (A) spathe inflating; (B) inflorescence fully flowering; (C) spathe trapping; (D & E) spathe abscising. A pairs of *Parastasia* beetles remain on appendix, the second male *P. nigripennis* (mating competitor) is forced to remain on the lower staminate flower zone. *Colocasiomyia* flies consume the pollen at the damaged staminate tissues; (F) pollen release adhere on *Colocasiomyia* flies but *Parastasia* beetles remain mating on appendix; **G**. Insects can escape from the moderately constricting spathe trapping device; **H – I.** *Parastasia* and *Chaloenus* beetle are consuming the interpistillar staminode; **J**. *Atheta* beetle is moving between the pistils; **K**. *Colocasiomyia* flies are sucking the interpistillar staminodes.

groups was significantly different (Friedman test, $p < 0.0003$). Pairwise comparisons (Wilcoxon test) showed significant different between *Chaloenus – Colocasiomyia, Colocasiomyia – Cycreon, Colocasiomyia – Parastasia, Colocasiomyia - Atheta*. However, during the latest field trip in July 2014, *Parastasia* was not found in all the five partially observed inflorescences (pistillate anthesis, 0800 – 1200). Species of *Colocasiomyia* was identified as *C.* aff. *bogneri; Chaloenus* beetle was identified as *C. latifrons*; *Parastasia* beetle was identified as *P. nigripennis*.

At the onset of pistillate anthesis, the scent released attracted firstly two visiting insects: *Colocasiomyia* flies and *Cycreon* beetles (0645). *Cycreon* beetles arrived and remained inside the lower chamber. Their activities were not seen due to their low visitation number (up to three individuals per inflorescence). *Colocasiomyia* flies mostly gathered on the staminate flower zone, highly active and moved rapidly from the appendix to the staminate flower zone. These flies mated (on appendix, staminate flower zone and inner spathe limb) and oviposited (on appendix, staminate and pistillate flower zone). Some of them sucked on the surface of interpistillar staminodes and inner spathe limb, fed on the liquid secreted from the stigma. By 0800, *Colocasiomyia* flies were less active and they shifted to the appendix (fed on the liquid secreted from the damaged appendix tissues gnawed by *Chaloenus* beetles and *P. nigripennis*) and inner spathe limb respectively.

Chaloenus beetles (0700) chewed the interpistillar staminodes, the appendix and the staminate flower zone. *Atheta* beetles (0725) mostly moved between the pistillate flowers and mated on the pistillate flower zone. An individual of *P. nigripennis* (0655) chewed the interpistillar staminodes on the upper pistillate flower zone. From the inner spathe limb, the *Parastasia*

beetle shook the spadix several times (0710 – 0720) and later moved upward to chew the appendix. By 0900, all insects were less active and mostly remained inside the lower chamber except *Chaloenus* beetles and the individual *P. nigripennis* remained on the appendix. However, within the other observed inflorescence, a pair of *P. nigripennis* was found mating on the appendix, and a second male *P. nigripennis* (mating competitor) remained on the staminate flower zone until the following day.

At the onset of staminate anthesis, *Atheta* beetles remained on the inner spathe (an individual) and staminate flower zone (an individual). When the spathe limb was abscised, the individual *Atheta* beetle that remains on the inner spathe limb was detached from the inflorescence. Another *Atheta* beetle was not found. An individual of *Cycreon* beetles left. No *Cycreon* beetle and *Atheta* beetle were observed to feed the pollen. From the second observed inflorescence, the first male *P. nigripennis* left the inflorescence. Slowly, the second *P. nigripennis* crawled to the appendix to mate with the female *P. nigripennis*, but pollen adhering was impossible. However, *Colocasiomyia* flies fed on the pollen extruded from the damaged staminate tissues (0510) or staminate flower zone during pollen release (0600 – 0635) and pollen adhered on their bodies. *Colocasiomyia* flies crawled onto *Chaloenus* beetles and *P. nigripennis,* transferred the adhered pollen onto both beetles. By 0715, all insects left and this marked the end of the anthesis of *S. roh* Ar2445.

Colocasiomyia flies are considered as the main pollinator whereas both *Chaloenus* beetles and *P. nigripennis* are considered as the opportunistic pollinator of *S. roh* Ar2445 as their visiting behaviours were found similar in *S. roh* Ar1240. *Cycreon* beetles and *Atheta* beetles are considered as visitors as their visitation number is low, were not observed to consume the

pollen and their visits were sporadic.

4.3.1.4 *Schismatoglottis baangongensis*

The total anthetic period of *S. baangongensis* was ca 29 hours: pistillate anthesis (wet pistils) started at 0400 and the staminate anthesis (pollen release) on the following day, at 0510 (**Figure 4.1, 4.6**). A day prior to anthesis (1830), some buds weakly loosened its spathe limb margin. At the onset of pistillate anthesis, the spathe loosened and a gap was formed along the spathe limb and lower spathe margin. A mild esteric scent was emitted (0400), turned intensified (0530), later reduced (0900) and ended (1000). By 0600, the spathe limb's colour turned from green to pale yellowish green, opened wide (ca 6.5 cm long x ca 3.3 cm wide), revealed a spathe limb opening (ca 2 cm long x ca 5 mm wide) and lower spathe inflated (ca 4 cm long x ca 2.3 cm wide). Within 0720 – 0930, a moderately constricting spathe trapping device was formed. It tightened its spathe limb (ca 3 cm wide) and lower spathe (ca 2 cm wide), spathe limb opening was fully enclosed (however, insects still could escape from the chamber). At the onset of staminate anthesis on the following day, the spathe limb started to abscise (0430) and fully abscised by 0500. White powdery pollen began to be released on the lower staminate flower zone (0510), and extended throughout the whole staminate flower zone (0530). After 35 – 40 days, the fruiting spathe splitted mediacopically and basiscopically to release the fruits from dawn onwards. The fruits and seeds were dispersed by unidentified red ants.

The mean ± SD of caught insects per inflorescence were *Chaloenus* beetles (4 ± 4), *Colocasiomyia* flies (227 ± 137), *Cycreon* beetles (54 ± 41), *Parastasia* beetles (1 ± 2) and *Atheta* beetles (3 ± 3) (**Table 4.2, Figure 4.3**). The number of visiting individuals among the

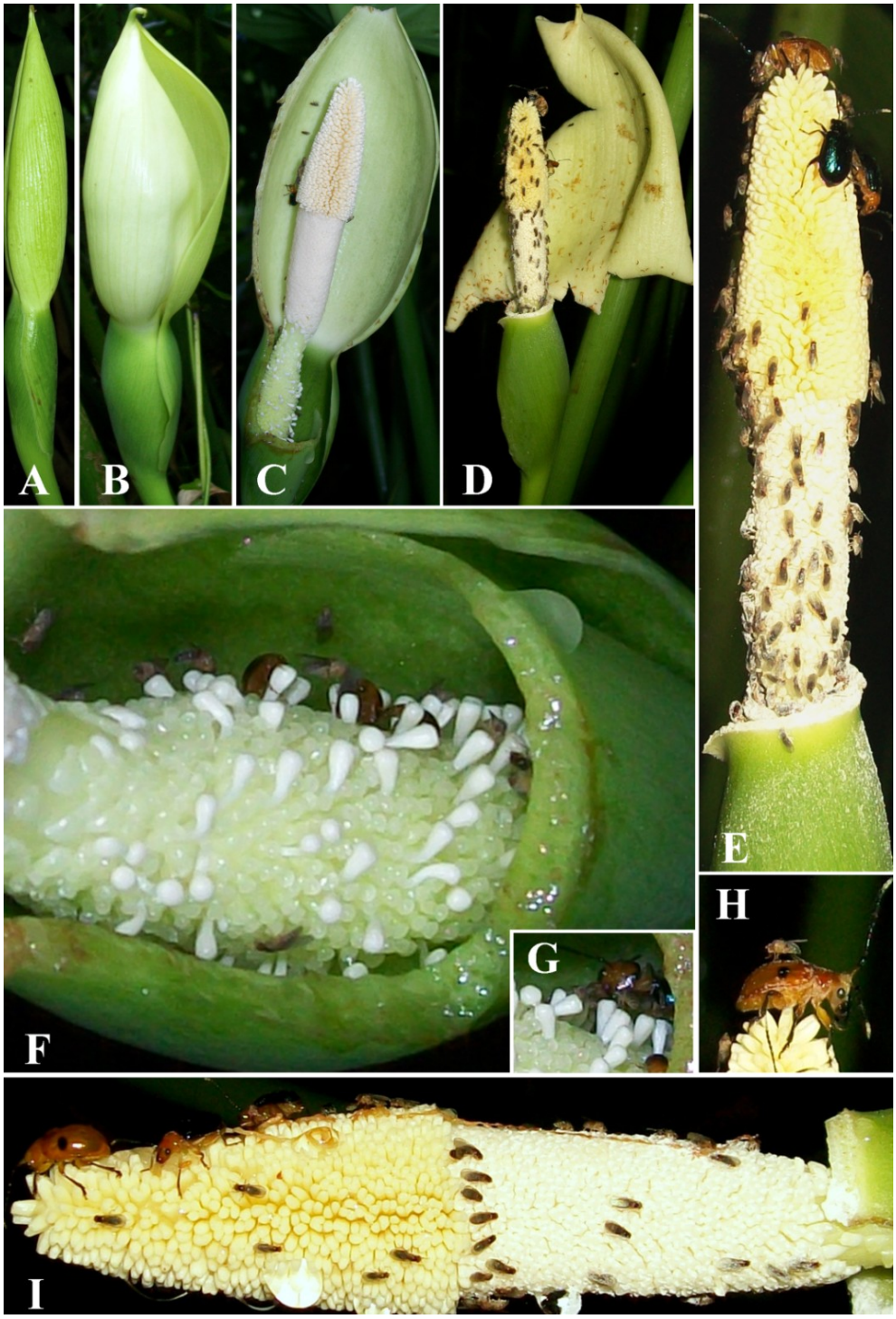

Figure 4.6. Flowering mechanisms and insect activities of *S. baangongensis*. **A – E.** Flowering sequence: (A) a day prior to anthesis; (B & C) pistillate anthesis; (D) spathe abscising. *Chaloenus* beetles remain on the appendix and *Colocasiomyia* flies remain on appendix and staminate flower zone; (E) *Colocasiomyia* flies consuming the pollen but *Chaloenus* beetles remain on the appendix; **F – G.** Insects are sucking (*Colocasiomyia* flies and *Cycreon* beetles) and chewing (*Chaloenus* beetle) the interpistillar staminodes; **H.** *Colocasiomyia* flies crawling onto *Chaloenus* beetle and removed the adhered pollen to *Chaloenus* beetle; **I.** An individual *Chaloenus* beetle laid its stranded eggs until the staminate flower zone.

insect groups was significantly different (Friedman test, $p < 0.000001$). Pairwise comparisons (Wilcoxon test) showed significant different between *Chaloenus – Colocasiomyia, Chaloenus – Cycreon, Colocasiomyia – Cycreon, Colocasiomyia – Parastasia, Colocasiomyia – Atheta*. Species of *Colocasiomyia* was identified as *C.* aff. *bogneri*; *Chaloenus* beetles were identified as *C. dohertyi, C. latifrons C. schawalleri* and *Chaloenus* sp. 1; *Parastasia* beetles were identified as *P. gestroi* and *P. nigripennis*.

At the onset of pistillate anthesis, the floral scent released attracted firstly two visited insects: *Colocasiomyia* flies and *Cycreon* beetles (0620). *Colocasiomyia* flies were highly active, moved freely within the inflorescence, mated on inner spathe, spadix (appendix, staminate and pistillate flower zone) and oviposited on appendix and staminate flower zone. These flies sucked on the surface of the interpistillar staminodes and inner spathe limb, fed on the liquid secreted from the stigma and damaged appendix tissues gnawed by *Chaloenus* beetles. *Cycreon* beetles mostly remained on lower spathe chamber to suck on the surface of the interpistillar staminodes and mated on the pistillate flower zone. Four *Chaloenus* beetles arrived (0720) to chew the interpistillar staminodes and appendix and mated on pistillate and appendix. *Atheta* beetles (1530) mostly moved through between the pistillate flowers. When the floral scent reduced (0900), all insects were less active and mostly remained inside the lower chamber until the following day except *Chaloenus* beetles which remained on the appendix.

At the onset of staminate anthesis, when the spathe limb was abscised, three individuals of *Atheta* beetles that remain on the inner spathe limb were detached from the inflorescence. During pollen release, another *Atheta* beetle departed from the pistillate flower zone without

pollen adhered. *Colocasiomyia* flies consumed the pollen on the whole staminate flower zone yet *Cycreon* beetles mostly consumed the pollen on the lower staminate flower zone. Pollen was fully adhered on *Colocasiomyia* flies and *Cycreon* beetles. *Chaloenus* beetles remained on the appendix thus pollen adhering was impossible except *Colocasiomyia* flies frequently crawling onto *Chaloenus* and transferred some of the adhered pollen to the *Chaloenus* beetles. Two *Chaloenus* beetles oviposited on appendix, an individual of *Chaloenus* beetle oviposited on the appendix and staminate flower zone. By 0615, floral scent was detected from a newly flowering *S. baangongensis* (pistillate anthesis), some *Colocasiomyia* flies and *Cycreon* beetles began to leave. *Chaloenus* beetles began to leave by 0635 and all insects left by 0900. From a total of 23 inflorescences (fully observed, partially observed and insect collecting), only three inflorescences (insect collecting) were found with 1 – 5 *Parastasia* beetles.

Colocasiomyia flies and *Cycreon* beetles are considered as the main pollinators. *Chaloenus* beetles are considered as the opportunistic pollinator of *S. baangongensis* as their visiting behaviours were found similar to *S. roh* Ar1240. *Parastasia* beetles are merely visitor as their visitation is sporadic. *Atheta* beetles are also visitors as no pollen adhering was seen.

4.3.1.5 *Schismatoglottis giamensis*

The total anthetic period of *S. giamensis* was ca 27 hours: pistillate anthesis (wet pistils) started at 0500 and the staminate anthesis (pollen release) on the following day, at 0600 (**Figure 4.1, 4.7**).

At the onset of pistillate anthesis, the lower spathe revealed a ca 3 mm marginal gap opening. A mild esteric scent was emitted (0500), turned intensified (0600), later reduced (0800) and

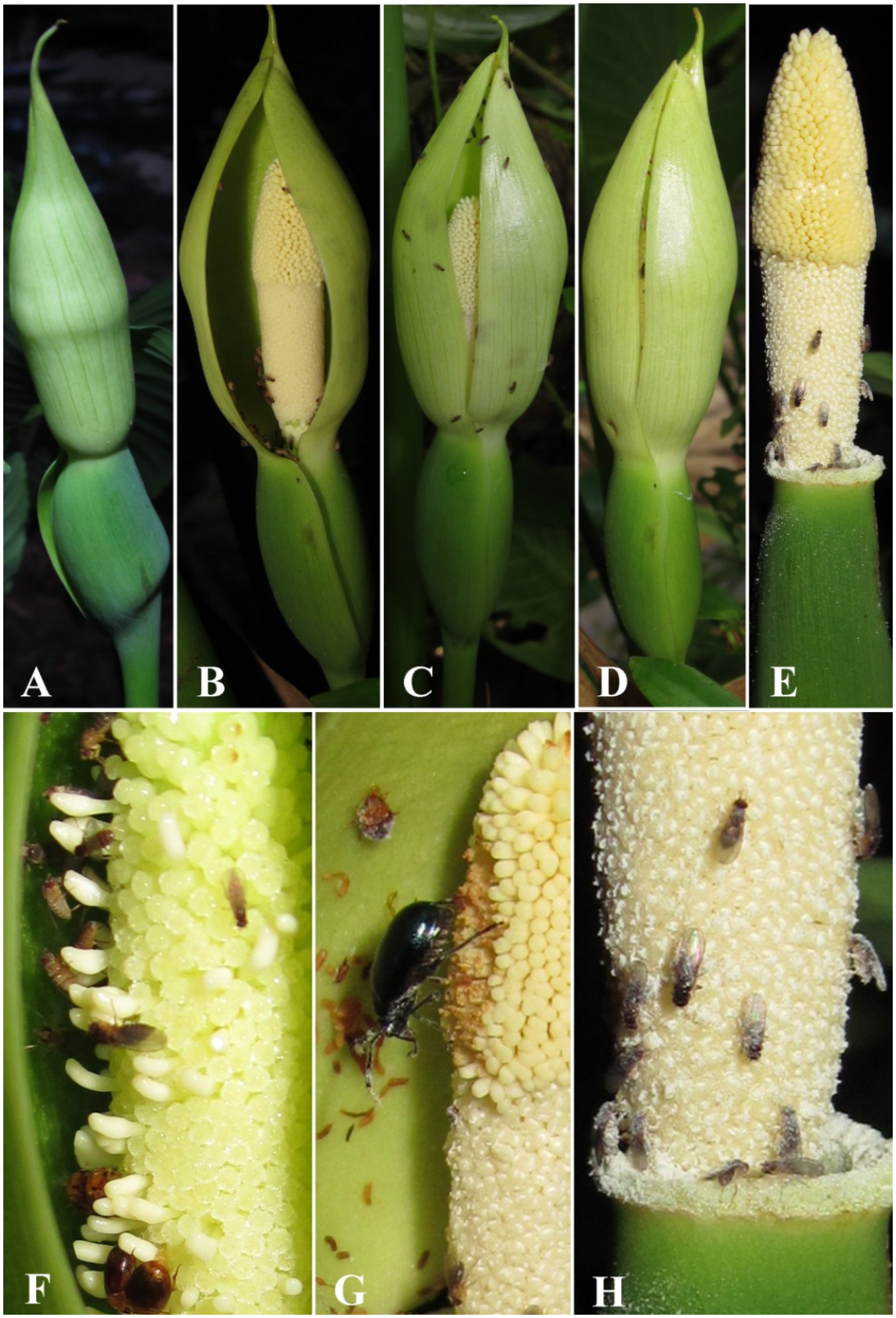

Figure 4.7. Flowering mechanisms and insect activities of *S. giamensis.* **A.** Spathe inflating; **B.** inflorescence fully flowering; **C – D.** Spathe trapping; **E**. Pollen release; **F.** *Colocasiomyia* flies and *Cycreon* beetles are sucking the interpistillar staminodes; **G.** *Chaloenus* beetle chews the appendix. Pollen adhere on individual *Cycreon* beetle that moving on the inner spathe limb; **H.** *Colocasiomyia* flies consume the pollen.

ended (1130). By 0640, the spathe limb's colour turned from green to pale yellowish green, opened wide (ca 5.5 cm long x ca 2.9 cm wide), revealed a spathe limb opening (ca 1.5 cm long x ca 4 mm wide) and lower spathe inflated (ca 3.5 cm long x ca 2 cm wide). Between 0730 – 0900, a moderately constricting spathe trapping device was formed. It tightened its spathe limb (ca 2.7 cm wide) and lower spathe (ca 1.8 cm wide), spathe limb opening was fully enclosed (however, insects still could escape from the chamber). At the onset of staminate anthesis on the following day, the spathe limb started to abscise (0540) and fully abscised by 0600. White powdery pollen began to be released from the lower staminate flower zone (0600), and extended throughout the whole staminate flower zone (0615). After 35 – 40 days, the fruiting spathe splitted and fruits were released from dawn onwards. The fruiting spathe senescence mechanism was not fully observed.

The mean ± SD of caught insects per inflorescence were *Chaloenus* beetles (3 ± 3), *Colocasiomyia* flies (127 ± 78), *Cycreon* beetles (23 ± 25), *Atheta* beetles (3 ± 4) and Pteromalid wasps (1 ± 3) (**Table 4.2, Figure 4.3**). The number of visiting individuals among the insect groups was significantly different (Friedman test, $p < 0.00001$). Pairwise comparisons (Wilcoxon test) showed significant different between *Chaloenus – Colocasiomyia, Chaloenus – Cycreon, Colocasiomyia – Atheta, Colocasiomyia* – Pteromalid, *Cycreon – Atheta* and *Cycreon* – Pteromalid. Species of *Colocasiomyia* was identified as *C.* aff. *bogneri*; *Chaloenus* beetles were identified as *C. latifrons*, *C. schawalleri, Chaloenus* sp. 1 and *Chaloenus* sp. 3.

At the onset of pistillate anthesis, the floral scent released attracted firstly two visiting insects: *Colocasiomyia* flies and *Cycreon* beetles (0640). *Colocasiomyia* flies were highly active,

moved freely within the inflorescence, mated on inner spathe limb and spadix (appendix, staminate and pistillate flower zone). These flies sucked on the surface of the interpistillar staminodes and inner spathe limb, fed on the liquid secreted from the stigma and damaged appendix tissues gnawed by *Chaloenus* beetles. *Cycreon* beetles mostly remained on lower spathe chamber to suck on the surface of the interpistillar staminodes and mated on pistillate flower zone. Two *Chaloenus* beetles arrived (0700) and chewed the interpistillar staminodes and terminal part of the appendix. When the floral scent reduced (0800), all insects were less active and mostly remained inside the lower chamber until the following day except *Chaloenus* beetles which remained on the appendix.

At the onset of staminate anthesis, *Colocasiomyia* flies consumed the pollen on the whole staminate flower zone yet *Cycreon* beetles mostly consumed the pollen on the lower staminate flower zone. Pollen was fully adhered on their bodies. *Chaloenus* beetles remained on the appendix to chew the appendix and laid eggs. Some pollen adhered on the head of an individual *Chaloenus* beetle when it positioned itself on the upper staminate flower zone to chew the appendix. By 0800, all insects left and this marked the end of the anthesis of *S. giamensis*. From a total of 16 inflorescences (fully observed, partially observed and insect collecting), two inflorescences (insect collecting) were found with by 3 – 9 Pteromalid wasps. *Atheta* beetles were absent during pollination investigations but it was found in independent sets of inflorescences bagged for insect identification.

Colocasiomyia flies and *Cycreon* beetles are considered as the main pollinators of *S. giamensis* as their visiting behaviours were found similar to *S. roh* Ar1240. *Chaloenus* beetles are considered as the opportunistic pollinators as they were destructive (gnawed the spadix)

and remained on appendix during pollen release but adhered pollen was found on an ndividual *Chaloenus* beetle. The sporadic Pteromalid wasps and *Atheta* beetles are considered as visitors.

4.3.1.6 *Schismatoglottis muluensis*

The total anthetic period of *S. muluensis* was ca 27 hours: pistillate anthesis (wet pistils) started at 0430 and the staminate anthesis (pollen release) on the following day, at 0530 (**Figure 4.1, 4.8**). A day prior to anthesis (2100), a weak reminiscent of esteric scent was detected from the buds and some buds loosened its lower spathe (ca 3 mm marginal gap opening). At the onset of pistillate anthesis, the esteric scent turned intensified (0500), later reduced (0930) and ended (1130). By 0430, 5 – 7 mm marginal gap was formed along the whole spathe. The spathe limb's colour turned from green to pale yellowish green, opened wide (0500) (ca 6.5 cm long x ca 2.5 cm wide) and lower spathe inflated (ca 4 cm long x ca 1.7 cm wide). Between 0645 – 0800, a moderately constricting spathe trapping device was formed. It tightened its spathe limb (ca 2.1 cm wide) and lower spathe (ca 1.5 cm wide), spathe limb opening was fully enclosed (however, insects still could escape from the chamber). At the onset of staminate anthesis on the following day, the spathe limb started to abscise (0345) and fully abscised by 0415. White powdery pollen began to be released on the lower staminate flower zone (0530), and extended throughout the whole staminate flower zone (0600). After 35 – 40 days, the fruiting spathe splitted acroscopically and mediacopically to release the fruits from dawn onwards. The fruits and seeds were dispersed by unidentified red and black ants.

The mean ± SD of caught insects per inflorescence were *Chaloenus* beetles (2 ± 2), *Colocasiomyia* flies (103 ± 31), *Cycreon* beetles (34 ± 22), *Parastasia* beetles (1 ± 1), *Atheta*

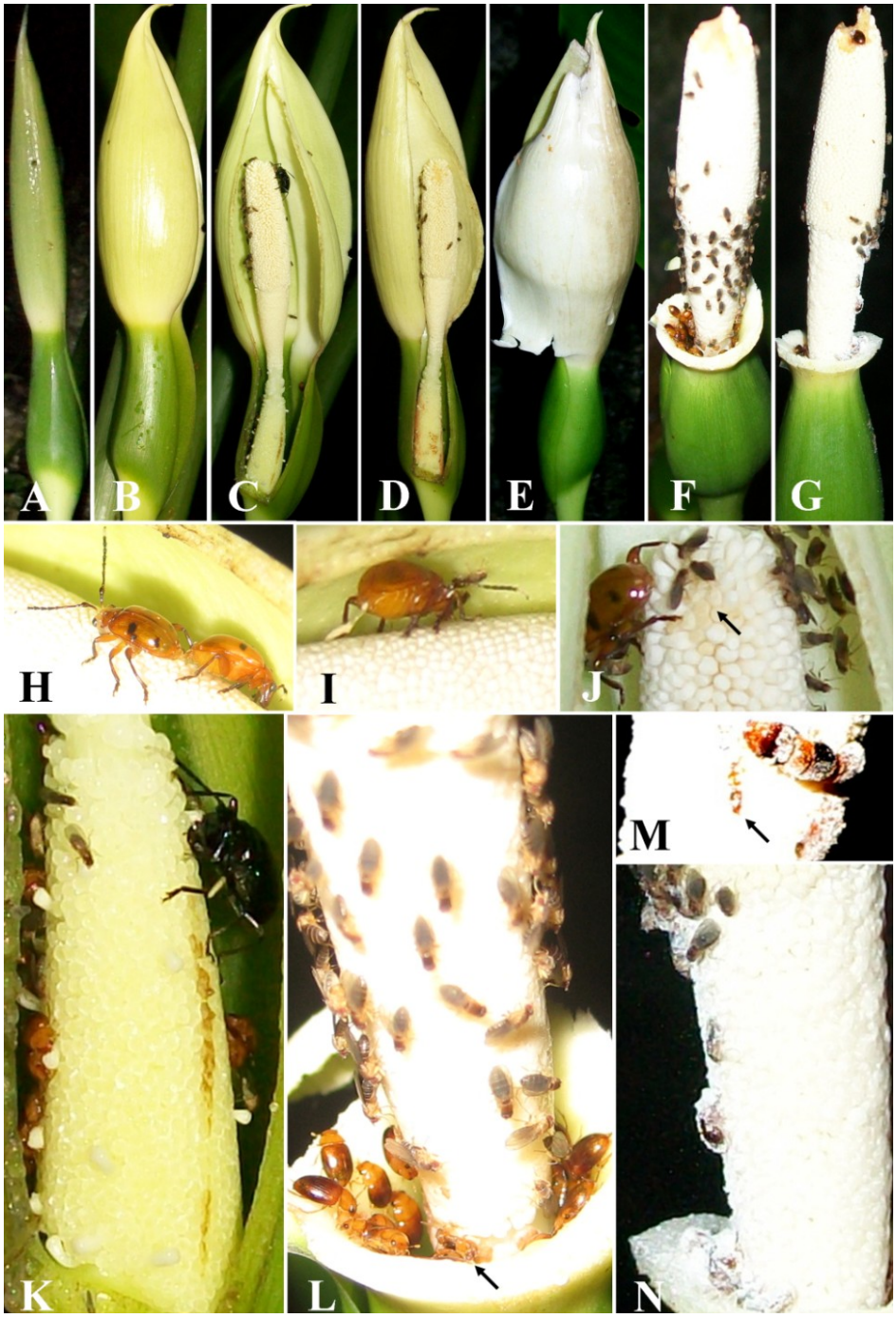

Figure 4.8. Flowering mechanisms and insect activities of *S. muluensis* M.Hotta. **A – G.** Flowering sequence: (A & B) spathe inflating; (C) inflorescence fully flowering; (D) spathe trapping, reddish colour on pistillate flower zone is damage caused by spathe cutting; (E) spathe abscising; (F) prior to pollen release, *Colocasiomyia* flies mostly remain on staminate flower zone, wherelse *Cycreon* beetles and *Atheta* beetles remain on the spathe constriction flower zone; (G) pollen release; **H – I.** *Chaloenus* beetles mate and lay eggs on the appendix; **J.** Black arrow indicate *Colocasiomyia* flies fed on the liquid secreted from damaged appendix tissues gnawed by *Chaloenus* beetles; **K.** Insects are sucking (*Colocasiomyia* flies and *Cycreon* beetles) or chewing (*Chaloenus* beetles) the interpistillar staminodes; **L.** Black arrow indicate present of *Atheta* sp.; **M.** Black arrow indicate pollen adhere on *Atheta* sp.; **N**. Pollen adhere on *Colocasiomyia* flies and *Cycreon* spp. that consume the pollen.

beetles (9 ± 6) and *Trigona* bees (1 ± 1) (**Table 4.2, Figure 4.3**). The number of visiting individuals among the insect groups was significantly different (Friedman test, $p < 0.0001$). Pairwise comparisons (Wilcoxon test) showed significant different between *Chaloenus – Colocasiomyia, Chaloenus – Cycreon, Colocasiomyia – Cycreon, Colocasiomyia – Parastasia, Colocasiomyia – Atheta*, *Colocasiomyia – Trigona* spp., *Cycreon – Parastasia, Cycreon – Atheta, Cycreon – Trigona* spp. and *Parastasia – Atheta*. Species of *Colocasiomyia* was identified as *C.* aff. *bogneri*; *Chaloenus* beetles were identified as *C. dohertyi* and *C. schawalleri*; *Parastasia* beetle was identified as *P. nigripennis*.

At the onset of pistillate anthesis, the floral scent released attracted firstly *Colocasiomyia* flies (0530). These flies were highly active, moving rapidly from the appendix to the staminate flower zone, flipped their wings to courtship and mated. *Colocasiomyia* flies also mated on the pistillate flower zone and inner spathe limb, sucked on the surface of the interpistillar staminodes and inner spathe limb, fed on the liquid secreted from the stigma and damaged appendix tissues gnawed by *Chaloenus* beetles. By 0630, *Cycreon* beetles arrived and sucked on the surface of the interpistillar staminodes and mated on pistillate flower zone. *Atheta* beetles arrived and mostly visited the pistillate flower zone. Two *Trigona* bees visited the spathe limb but left after a minute. *Chaloenus* beetles chewed the appendix, interpistillar staminodes and spathe limb; mated and laid eggs on the appendix. When the floral scent reduced (0930), all insects less active and mostly remained inside the lower chamber until the following day except *Chaloenus* beetles which remained on the appendix.

At the onset of anthesis, the spathe limb is slightly pallid brownish white and all *Chaloenus* beetles left the inflorescence (0300). *Colocasiomyia* flies mostly gathered on the staminate

flower zone and a few on the appendix. *Cycreon* beetles and *Atheta* beetles gathered on inner part of the spathe constriction flower zone. During pollen release, *Colocasiomyia* flies consumed pollen on the staminate flower zone. *Cycreon* beetles and *Atheta* beetles consumed the accumulated fallen pollen on inner part of the spathe constriction flower zone. *Cycreon* beetles further crawled up to staminate flower zone to consume the pollen. By 0630, all *Colocasiomyia* flies left the inflorescences. *Cycreon* beetles and *Atheta* beetles were the last to leave (0730) and this marked the end of the staminate anthesis of *S. muluensis*. From a total of 26 inflorescences (fully observed, partially observed and insect collecting), only two inflorescences (from insect collecting) were visited by an individual of *P. nigripennis* respectively.

Colocasiomyia flies, *Cycreon* beetles and *Atheta* beetles are considered as the main pollinators of *S. muluensis* as their visiting behavious were found similar in *S. roh* Ar1240. *Trigona* bees are visitors as these bees never visited the pistillate flower zone. *Chaloenus* beetles are considered as visitors that mainly gnawed on different inflorescence parts and left prior to pollen release. *Parastasia* beetles are visitors as their visitations are sporadic.

4.3.1.7 *Schismatoglottis pseudoniahensis*

The total anthetic period of *S. pseudoniahensis* was ca 26 hours: pistillate anthesis (wet pistils) started at 0430 and the staminate anthesis (pollen release) on the following day, at 0515 (**Figure 4.1, 4.9**). At the onset of pistillate anthesis, the lower spathe firstly loosened (0430, ca 2 mm marginal gap), followed by spathe limb (0600, ca 3 mm marginal gap). A mild esteric floral scent was emitted (0430), turned intensified (0620), later reduced (1100) and ended (1600). By 0630, a ca 5 mm marginal gap was formed along the whole spathe. The spathe

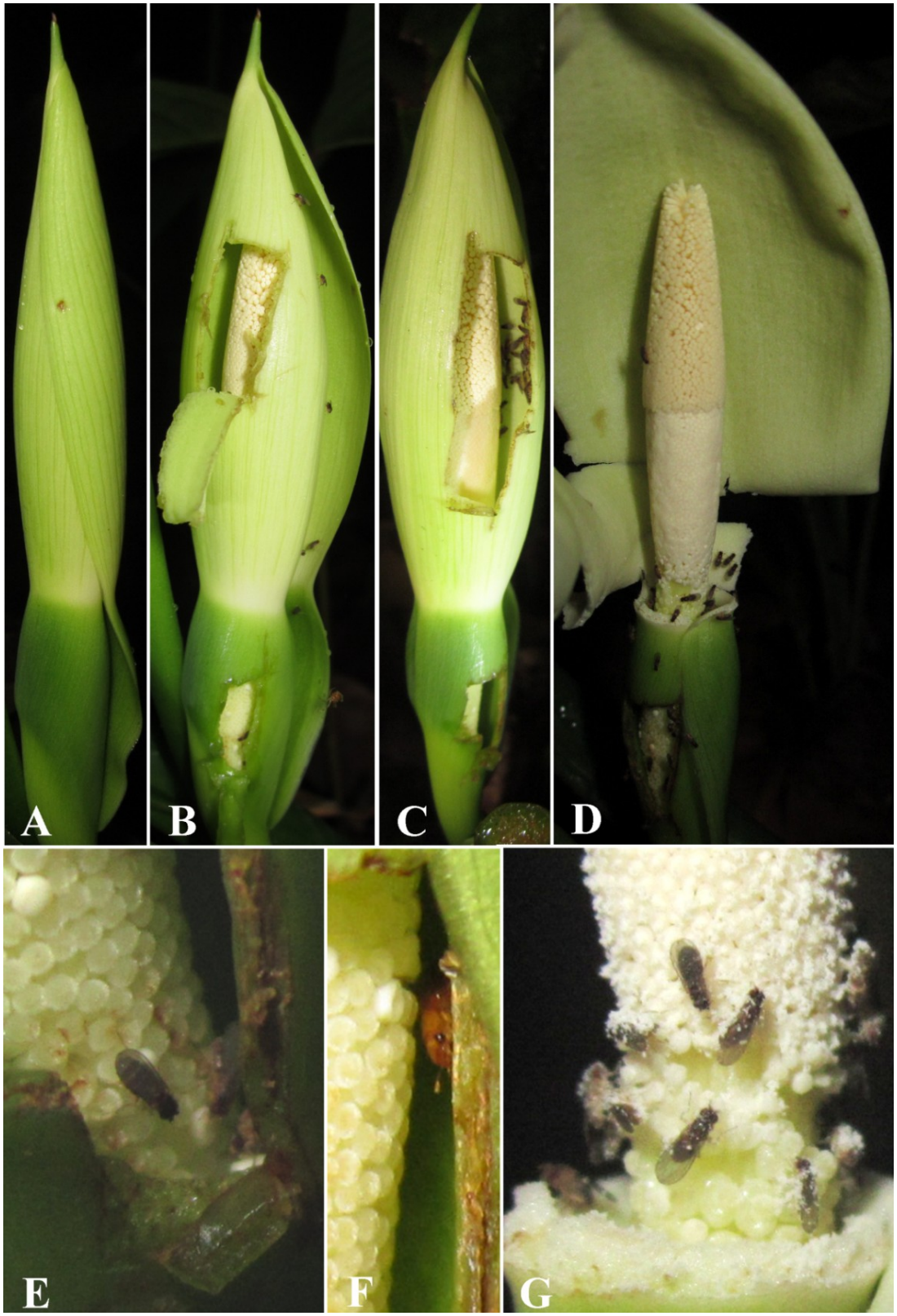

Figure 4.9. Flowering mechanisms and insect activities of *S. pseudoniahensis*. **A – D.** Flowering sequence: (A) spathe inflating; (B) inflorescence fully flowering; (C) spathe trapping; (D) spathe abscising and pollen release; **E.** *Colocasiomyia* flies are sucking the interpistillar staminodes; **F**. *Cycreon* beetle visiting the pistillate flower zone; **G.** Pollen adhere on *Colocasiomyia* flies.

limb's colour turned from green to creamy yellowish green, opened wide (ca 6 cm long x ca 2.3 cm wide) and lower spathe inflated (ca 3.5 cm long x ca 1.6 cm wide). Betwee 0700 – 0800, a moderately constricting spathe trapping device was formed. It tightened its spathe limb (ca 2 cm wide) and lower spathe (ca 1.5 cm wide), marginal gap of the spathe was fully enclosed (however, insects still could escape from the chamber). At the onset of staminate anthesis on the following day, the spathe limb started to abscise (0420) and fully abscised by 0435. White powdery pollen began to be released on the lower staminate flower zone (0515), and extended throughout the whole staminate flower zone (0530). After 35 – 40 days, the fruiting spathe splitted acroscopically to release the fruits from dawn onwards. The fruits and seeds were dispersed by unidentified red ants.

The mean ± SD of caught insects per inflorescence were *Colocasiomyia* flies (46 ± 41), *Cycreon* beetles (1 ± 1) and *Atheta* beetles (1 ± 1). The number of visiting individuals among the insect groups was significantly different (Friedman test, $p < 0.01$) (**Table 4.2, Figure 4.3**). The floral scent released attracted firstly *Colocasiomyia* flies (0615). These flies mostly gathered on staminate flower zone, highly active and moving rapidly from the appendix to the staminate flower zone, flipped their wings to courtship and mated. *Colocasiomyia* flies also mated on the pistillate flower zone and inner spathe limb. These flies sucked on the surface of the interpistillar staminodes and inner spathe limb, fed on the liquid secreted from the stigma. By 0720, *Colocasiomyia* flies were less active and remained on inner spathe limb, later moved to lower chamber (1100) until the following day. Two *Cycreon* beetles arrived (0635) to suck on the surface of interpistillar staminodes and remained inside the lower chamber.

At the onset of staminate anthesis, *Colocasiomyia* flies shifted to the lower staminate flower zone to consume the pollen and pollen adhered on their body. Prior to leaving, these flies clean themselves (similar in *S. calyptrata*) (0540). Due to low numbers of *Cycreon* beetles, their activities were not observed. By 0630, all insects left the inflorescence and this marked the end of the anthesis of *S. pseudoniahensis*. From a total of eight inflorescences (fully observed and insect collecting), only one inflorescence was visited by an individual of *Atheta* beetle, and four inflorescences were visited by the *Cycreon* beetles. *Colocasiomyia* flies are considered as the main pollinator of *S. pseudoniahensis* as their visiting behaviours were found similar to *S. calyptrata.* The sporadic individual of *Atheta* beetle is considered as visitor. Status of *Cycreon* beetle is unclear as the number was low (up to two individuals per inflorescence) and they were found sporadically.

4.3.1.8 *Schismatoglottis adducta*

The total anthetic period of *S. adducta* was ca 26 hours: pistillate anthesis (wet pistils) started at 0500 and the staminate anthesis (pollen release) on the following day, at 0545 (**Figure 4.1, 4.10**). At the onset of pistillate anthesis, lower spathe loosened to ca 2 mm marginal gap opening but spathe limb remained tightened (0500 – 0530). A mild esteric scent was emitted (0500), turned intensified (0630), later reduced (0800) and ended (1050). By 0700, spathe limb's colour turned from green to pale yellowish green, opened wide (ca 5 cm long x ca 2.2 cm wide) and lower spathe inflated (ca 3.2 cm long x ca 1.3 cm wide) (0700). Between 0730 – 0900, a moderately constricting spathe trapping device was formed. It tightened its spathe limb (ca 1.6 cm wide) and lower spathe (ca 1.1 cm wide), marginal gap of the spathe was fully enclosed. At the onset of staminate anthesis on the following day, the spathe limb started to abscise (0440) and fully abscised by 0530. White powdery pollen began to be released on the

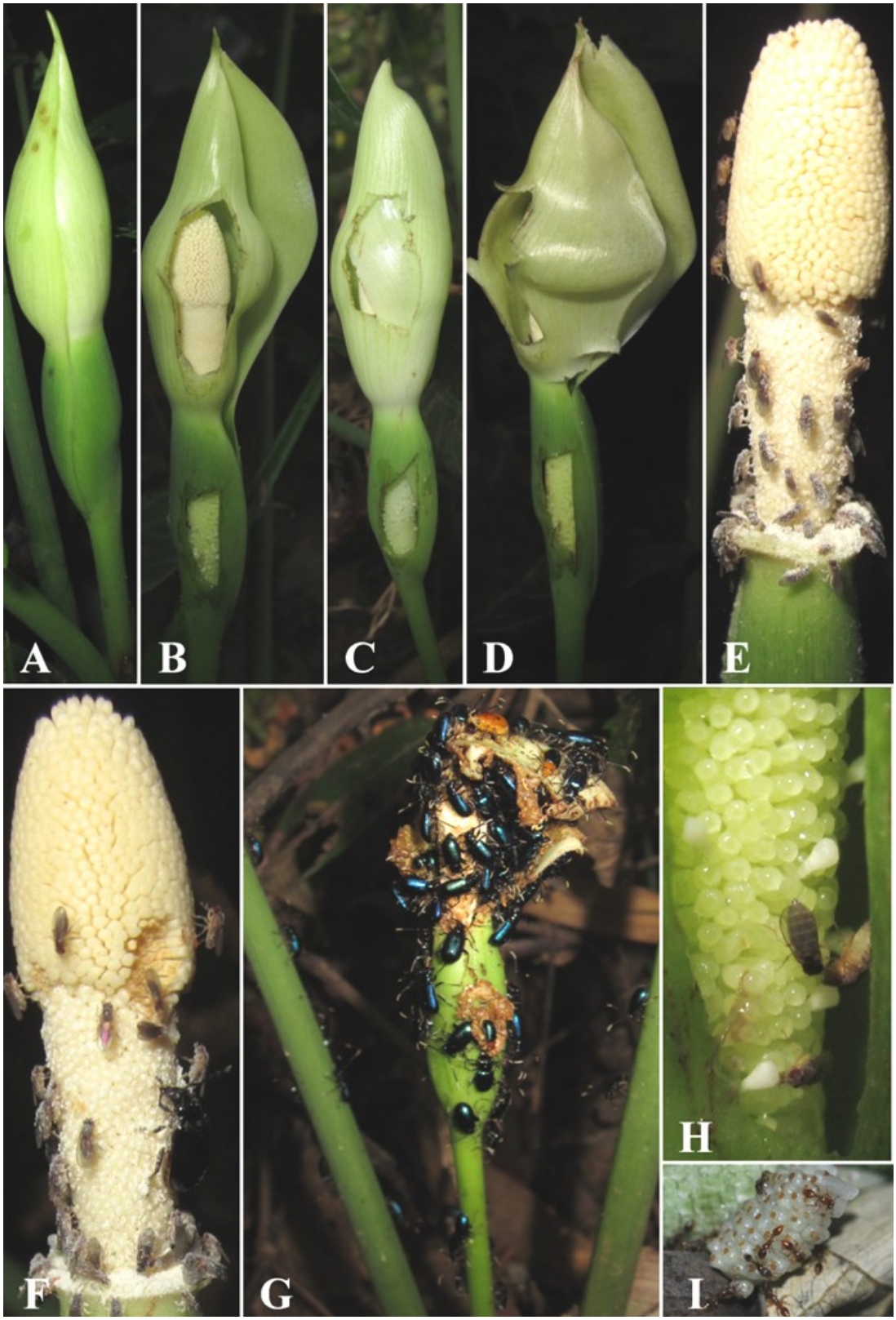

Figure 4.10. Flowering mechanisms and insect activities of *S. adducta*. **A – F.** Flowering sequence: (A) spathe inflating; (B) fully flowering; (C) spathe trapping; (D) spathe abscising; (E) pollen adhere on *Colocasiomyia* flies, *Cycreon* beetles and (F) an individual *Chaloenus* beetle; **G.** *Chaloenus* beetles damaging the solitary inflorescence; **H.** *Colocasiomyia* flies sucking the interpistillar staminodes; **I.** Unidentified red ants disperses the dehisced fruits.

lower staminate flower zone (0530), and extended throughout the whole staminate flower zone (0545). After 35 – 40 days, the fruiting spathe splitted acropetally and basiscopically to release the fruits from dawn onwards. The fruits and seeds were dispersed by unidentified red ants.

The mean ± SD of caught insects per inflorescence were *Chaloenus* beetles (4 ± 8), *Colocasiomyia* flies (77 ± 31), *Cycreon* beetles (10 ± 5) and *Atheta* beetles (1 ± 1) (**Table 4.2, Figure 4.3**). The number of visiting individuals among the insect groups was significantly different (Friedman test, $p < 0.0002$). Pairwise comparisons (Wilcoxon test) showed significant differences between insects of *Chaloenus – Colocasiomyia, Colocasiomyia – Cycreon, Colocasiomyia – Atheta* and *Cycreon – Atheta*. Species of *Colocasiomyia* was identified to *C.* aff. *bogneri; Chaloenus* beetles were *C. schawalleri, C. latifrons* and *Chaloenus* sp. 3. *Colocasiomyia* was identified to *C.* aff. *bogneri; Chaloenus* beetles were *C. schawalleri, C. latifrons* and *Chaloenus* sp. 3.

At the onset of pistillate anthesis, the floral scent released attracted firstly *Colocasiomyia* flies (0615). These flies were highly active, moving rapidly from the appendix to the staminate flower zone and mated on inner spathe and spadix zones (appendix, staminate and pistillate flower zone). *Colocasiomyia* flies sucked on the surface of the interpistillar staminodes and inner spathe limb, fed on the liquid secreted from the stigma and damaged appendix tissues gnawed by *Chaloenus* beetles. *Cycreon* beetles mostly remained on the lower spathe chamber to suck on the surface of the interpistillar staminodes and mated on the pistillate flower zone. An individual of *Chaloenus* beetle arrived (0710) and chewed the appendix. When the floral

scent reduced (0800), all insects were less active and mostly remained inside the lower chamber until the following day except *Chaloenus* beetles which remained on the appendix.

At the onset of staminate anthesis, *Colocasiomyia* flies consumed the pollen on the whole staminate flower zone while *Cycreon* beetles mostly consumed the pollen on the lower staminate flower zone. Pollen was fully adhered on *Colocasiomyia* flies and *Cycreon* beetles. The pollen also adhered on one individual *Chaloenus* beetle that remained on staminate flower zone, and this beetle later moved to the appendix. By 0605, the individual *Chaloenus* beetle left the inflorescence, followed by *Colocasiomyia* flies (0610 – 0635), and *Cycreon* beetles (0645 – 0700). From a total of 11 inflorescences (fully observed and insect collecting), four fully observed inflorescences were visited by one *Chaloenus* beetle and one inflorescence (insect collecting) was visited by one of *Atheta* beetle.

Colocasiomyia flies and *Cycreon* beetles are considered as the main pollinators of *S. sungairayaensis* as their visiting behaviours were found similar in *S. roh* Ar1240. *Chaloenus* beetle is regarded as the opportunistic pollinator as they left with adhered pollen but their visitation is sporadic. *Atheta* beetle is considered as visitor as they visit sporadically the inflorescence of this species.

4.3.1.9 *Schismatoglottis caesia*

The total anthetic period of *S. caesia* was ca 26.5 hours: pistillate anthesis (wet pistils) started at 0500 and the staminate anthesis (pollen release) on the following day, at 0550 (**Figure 4.1, 4.11**). At the onset of pistillate anthesis, lower spathe loosened to ca 2 mm marginal gap opening but the spathe limb remained tightened (0510 – 0615). A mild esteric scent was

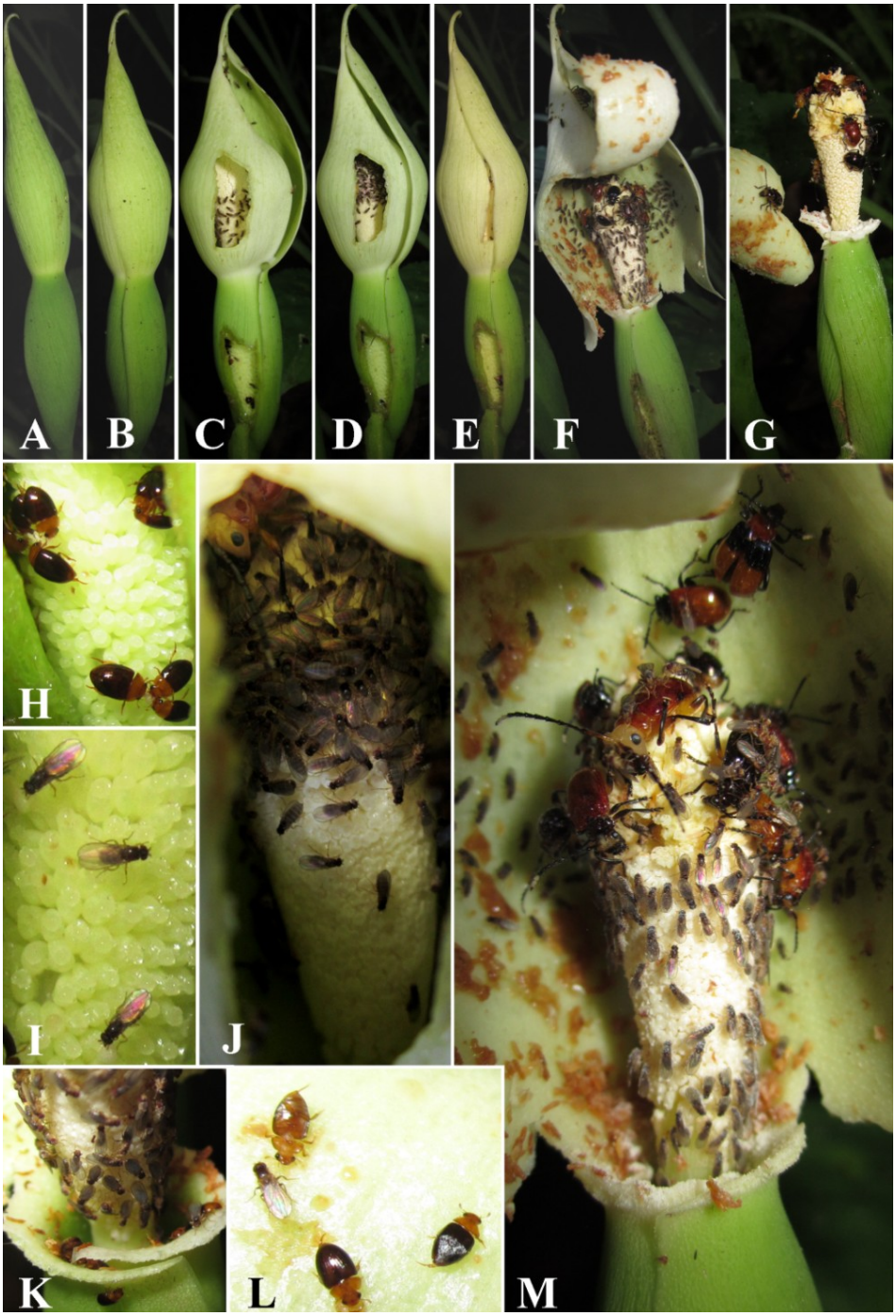

Figure 4.11. Flowering mechanisms and insect activities of *S. caesia*. **A – G.** Flowering sequence: (A – B) spathe inflating; (C) inflorescence fully flowering, *Colocasiomyia* flies mostly courtship (staminate flower zone) and sucking the interpistillar staminodes; (D) *Colocasiomyia* flies moved to appendix to fed on the liquid secreted from the damaged appendix tissues gnawed by *Chaloenus* beetles; (E) Spathe trapping; (F) Spathe abscising; (G) Some *Chaloenus* beetles were forced to remain on staminate flower zone; **H – I.** *Cycreon* beetles and *Colocasiomyia* flies are sucking the interpistillar staminodes; **J.** Zoom in image of (D); **K.** *Colocasiomyia* flies and *Cycreon* beetles waiting for pollen release; **L.** *Cycreon* beetles clean the adhered pollen by turning their body and contacting their elytra on the wet surface of inner spathe limb; **M.** *Colocasiomyia* flies crawling onto and removed the adhered pollen to *Chaloenus* beetles.

emitted (0500), turned intensified (0630), later reduced (0820) and ended (1400). By 0700, spathe limb's colour turned from green to pale yellowish green, opened wide (ca 7.0 cm long x ca 3.8 cm wide) and lower spathe inflated (ca 4.8 cm long x ca 1.8 cm wide). Between 0730 – 0900, a moderately constricting spathe trapping device was formed. It tightened its spathe limb (ca 3 cm wide) and lower spathe (ca 1.6 cm wide), marginal gap of the spathe was fully enclosed (however, insects still could escape from the chamber). At the onset of staminate anthesis on the following day, the spathe limb started to abscise (0500) and fully abscised by 0525. White powdery pollen began to be released on the lower staminate flower zone (0550), and extended throughout the whole staminate flower zone (0620). After 35 – 40 days, the fruiting spathe splitted acroscopically to release the fruits from dawn onwards. The fruits and seeds were dispersed by unidentified red ants.

The mean ± SD of caught insects per inflorescence were *Chaloenus* beetles (20 ± 18), *Colocasiomyia* flies (436 ± 82) and *Cycreon* beetles (81 ± 16) (**Table 4.2, Figure 4.3**). The number of visiting individuals among the insect groups was not significantly different (Friedman test, $p > 0.05$). The species of the *Chaloenus* was identified as *C. latifrons*. The floral scent released attracted firstly *Colocasiomyia* flies (0700). These flies were highly active, moved rapidly from the appendix to the staminate flower zone and mated on the inner spathe and staminate flower zone. *Colocasiomyia* flies sucked on the surface of the interpistillar staminodes and inner spathe limb, fed on the liquid secreted from the stigma and damaged appendix tissues gnawed by *Chaloenus* beetles. *Cycreon* beetles (0715) mostly remained on the lower spathe chamber to suck on the surface of the interpistillar staminodes and mated on the pistillate flower zone. *Chaloenus* beetles arrived (0740) and chewed the interpistillar staminodes and appendix. They also mated on staminate flower zone. When the

floral scent reduced (0820), all insects were less active and mostly remained inside the lower chamber until the following day except *Chaloenus* beetles which remained on the appendix.

At the onset of staminate anthesis, most *Colocasiomyia* flies shifted to the staminate flower zone and consumed the pollen during pollen release (0550 – 0620). Pollen was fully adhered onto their bodies. Prior to leaving (0630 – 0740), these flies moved to the appendix to clean themselves (similar in *S. calyptrata*), crawled onto *Chaloenus* beetles and passed the adhered pollen onto the bodies of *Chaloenus* beetles. *Chaloenus* beetles gathered on the appendix but owing to the crowded space, some *Chaloenus* beetles were forced to remain on the staminate flower zone and pollen was adhered onto their bodies. When the spathe limb fully abscises, spathe limb was sometimes seen hanging on the tip of the appendix. At this moment, *Cycreon* beetles removed the pollen by turning their body and contacting their elytra on the wet surface of inner spathe limb. *Cycreon* beetles also used their fore legs to remove the pollen from their head. By 0730, all insects left and this marked the end of the anthesis of *S. caesia.* However, *Chaloenus* beetles were found to leave a separate inflorescence by 1100. *Colocasiomyia* flies and *Cycreon* beetles are considered as the main pollinators and *Chaloenus* beetles are considered as the opportunistic pollinator of *S. caesia* as their visiting behaviours were found to be similar to *S. roh* Ar1240.

4.3.1.10 *Schismatoglottis pantiensis*

The total anthetic period of *S. pantiensis* was ca 26 hours: pistillate anthesis (wet pistils) started at 0500 and the staminate anthesis (pollen release) on the following day, at 0530 (**Figure 4.1, 4.12**). Two to three days before the anthesis, the upper marginal spathe limb slightly loosened. At the onset of pistillate anthesis, the spathe limb and lower spathe loosened

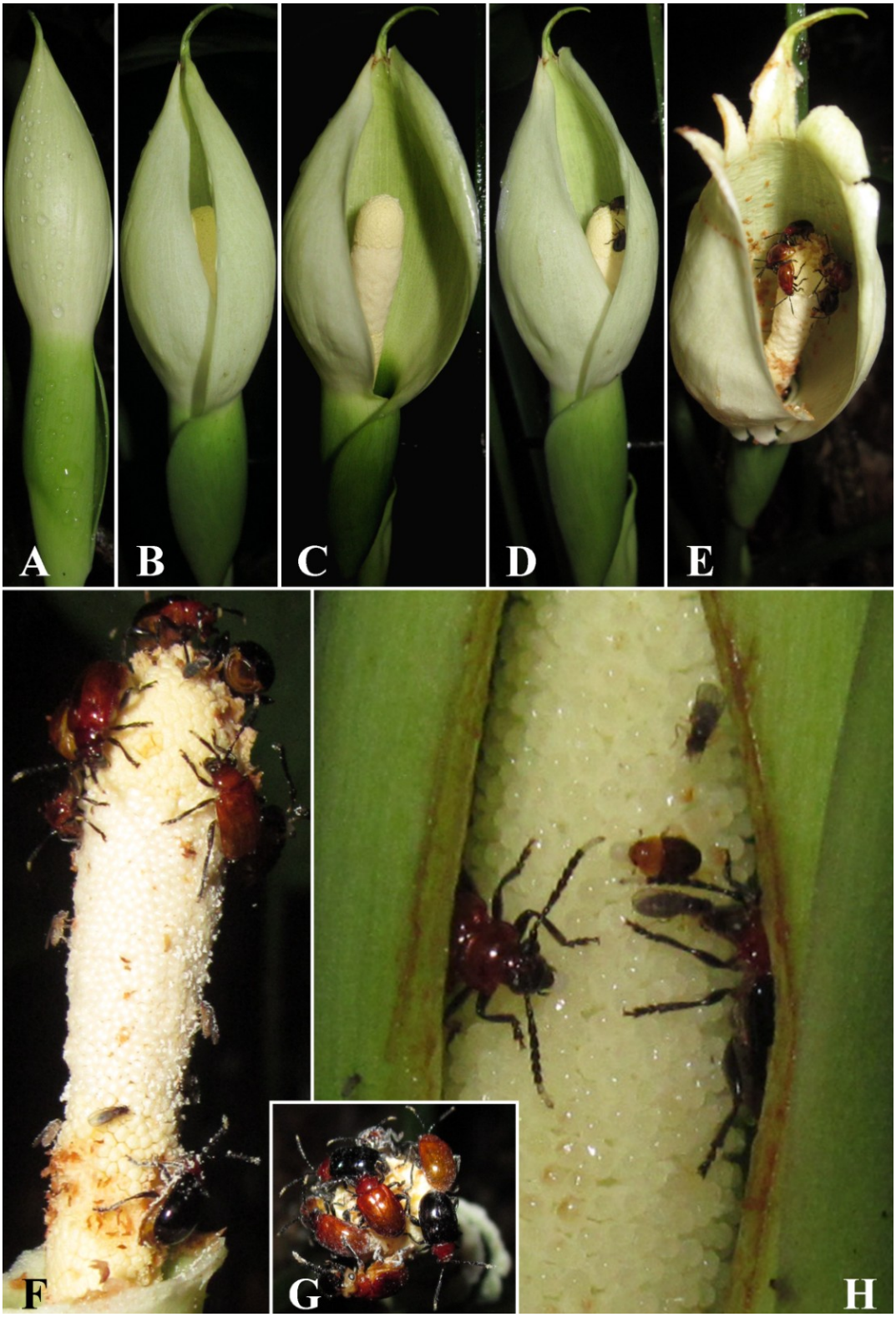

Figure 4.12. Flowering mechanisms and insect activities of *S. pantiensis*. **A – F.** Flowering sequence: (A & B) spathe inflating; (C) inflorescence fully flowering; (D) spathe trapping; (E) spathe abscising; (F) pollen release and an individual *Chaloenus* beetle was forced to move to interstice; **G.** Pollen adhere on *Chaloenus* beetles that competing for the space on the appendix; **H.** Insects are sucking (*Colocasiomyia* flies and *Cycreon* beetles) or chewing (*Chaloenus* beetles) the interpistillar staminodes.

to ca 2 mm and ca 3 mm marginal gap opening respectively (0500 – 0530). A mild esteric scent was emitted (0500), turned intensified (0615), later reduced (0730) and ended (1200). By 0700, the spathe limb's colour turned from green to pale yellowish green, opened wide (ca 7 cm long x ca 2.7 cm wide), revealed a spathe limb opening (ca 7 cm long x ca 1.2 cm wide) and lower spathe inflated (ca 5 cm long x ca 2 cm wide). From 0730 – 0830, a moderately constricting spathe trapping device was formed. It tightened its spathe limb (ca 3 cm wide) and lower spathe (ca 1.8 cm wide), spathe limb opening was fully enclosed. At the onset of staminate anthesis on the following day, the spathe limb started to abscise (0430) and fully abscised by 0450. White powdery pollen began to be released on the lower staminate flower zone (0530), and extended throughout the whole staminate flower zone (0550). After 35 – 40 days, the fruiting spathe splitted and fruits were released from dawn onwards. The fruiting spathe senescence mechanism was not fully observed.

The mean ± SD of caught insects per inflorescence were *Chaloenus* beetles (11 ± 5), *Colocasiomyia* flies (40 ± 19), *Cycreon* beetles (7 ± 3) (**Table 4.2, Figure 4.3**). The number of visiting individuals among the insect groups was not significantly different (Friedman test, $p > 0.05$). The species of the *Chaloenus* beetle was identified to *C. latifrons*. The floral scent released attracted firstly *Colocasiomyia* flies (0630). These flies were highly active, moved rapidly from the appendix to the staminate flower zone and mated on staminate flower zone. *Colocasiomyia* sucked on the surface of the interpistillar staminodes and inner spathe limb, fed on the liquid secreted from the stigma. *Cycreon* beetles (0700) mostly remained on the lower spathe chamber to suck on the surface of the interpistillar staminodes. *Chaloenus* beetles (0700 – 0730) chewed the interpistillar staminodes and appendix. They also fought on the lower staminate flower zone. When the floral scent reduced (0730), all insects were less

active and mostly remained inside the lower chamber until the following day except *Chaloenus* beetles remained on the appendix. However, due to the crowded space on the appendix, a few of *Chaloenus* beetles were forced to remain on the interstice.

At the onset of staminate anthesis, *Colocasiomyia* flies fed the pollen on the staminate flower zone and *Cycreon* beetles fed on the fallen pollen on the interstice. Pollen was fully adhered on both insects. Pollen also adhered on *Chaelonus* beetles that remained on interstice and when these beetles crawled up to appendix, it transferred the pollen to other *Chaloenus* beetles. Within 0600 – 0620, all *Colocasiomyia* flies and *Cycreon* beetles left. *Chaloenus* beetles left by 0700.

Colocasiomyia flies and *Cycreon* beetles were considered as the main pollinators of *S. pantiensis* as their visiting behaviours were found similar to *S. roh* Ar1240. *Chaloenus* beetles are considered as the opportunistic visitor as adhered pollen was found on their bodies when they competed for the crowded space on the appendix.

4.3.1.11 *Schismatoglottis laxipistillata*

The total anthetic period of *S. laxipistillata* was ca 28.5 hours: pistillate anthesis (wet pistils) started at 0430 and the staminate anthesis (pollen release) on the following day, at 0610 (**Figure 4.1, 4.13**). At the onset of pistillate anthesis, lower spathe loosened to ca 1 mm marginal gap opening but the spathe limb remained tightened (0500 – 0540). A mild esteric scent was emitted (0430), turned intensified (0630), later reduced (0900) and ended (1430). By 0840, spathe limb's colour turned from green to pale yellowish green, opened wide (ca 6.5 cm long x ca 3.5 cm wide) and lower spathe inflated (ca 4.2 cm long x ca 2 cm wide). From

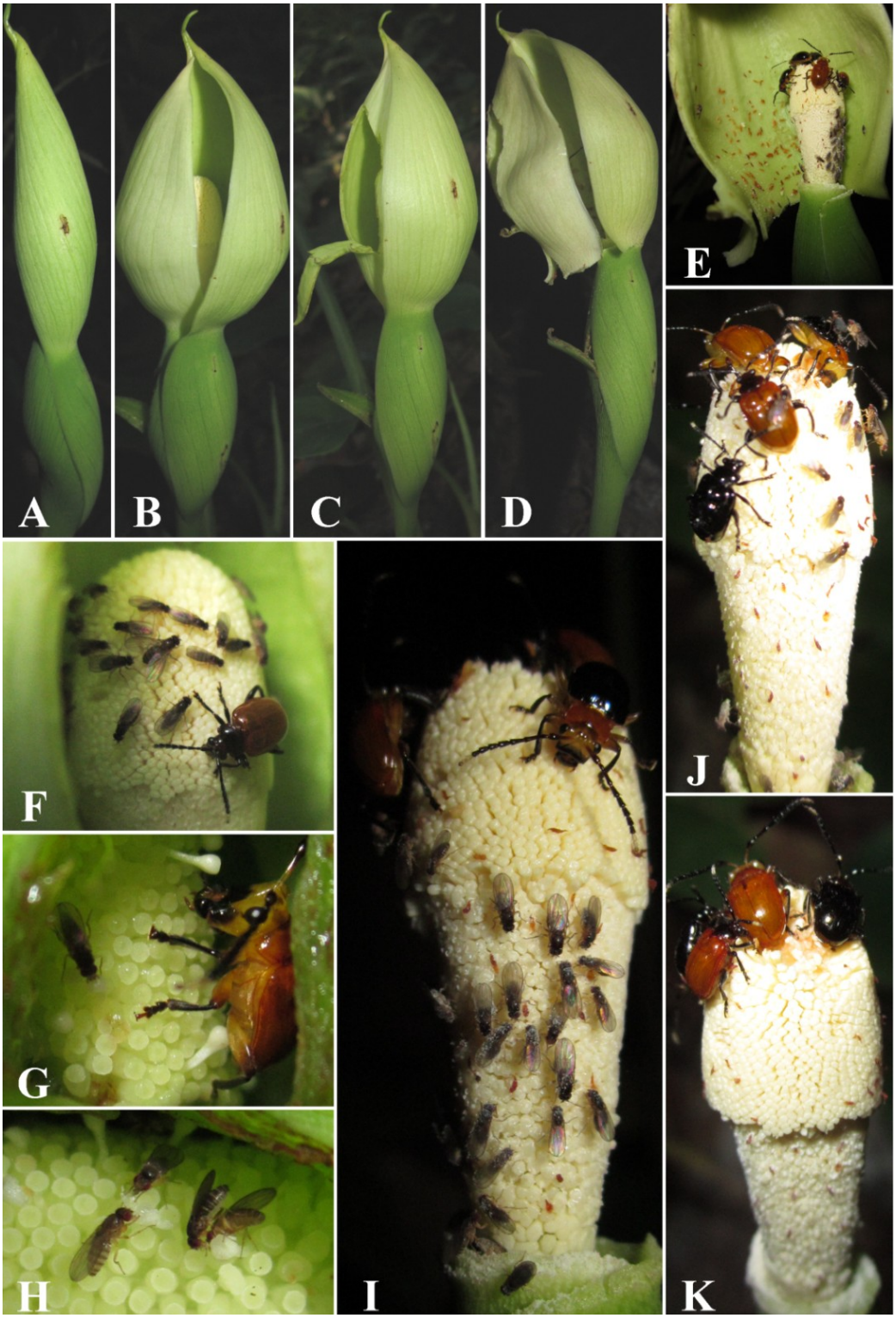

Figure 4.13. Flowering mechanisms and insect activities of *S. laxipistillata*. **A – E.** Flowering sequence: (A) spathe inflating; (B) inflorescence fully flowering; (C) spathe trapping; (D) spathe abscising; (E) pollen release; F. *Colocasiomyia* flies and *Chaloenus* beetle are visiting the appendix; **G – H**. Insects are sucking (*Colocasiomyia* flies and *Cycreon* beetles) or chewing (*Chaloenus* sp.) the interpistillar staminodes; **I.** *Colocasiomyia* flies consuming the pollen; **J.** *Colocasiomyia* flies crawling onto *Chaloenus* beetles and removed the adhered pollen to *Chaloenus* beetles; **K.** *Chaloenus* beetles lay eggs on the appendix.

0840 – 1000, a moderately constricting spathe trapping device was formed. It tightened its spathe limb (ca 2.5 cm wide) and lower spathe (ca 1.8 cm wide), marginal gap of the spathe was fully enclosed. At the onset of staminate anthesis on the following day, the spathe limb started to abscise (0600) and fully abscised by 0630. White powdery pollen began to be released on the lower staminate flower zone (0610), and extended throughout the whole staminate flower zone (0625). After 35 – 40 days, the fruiting spathe splitted and fruits were released from dawn onwards. The fruiting spathe senescence mechanism was not fully observed.

The mean ± SD of caught insects per inflorescence were *Chaloenus* beetles (4 ± 2) and *Colocasiomyia* flies (28 ± 13) (**Table 4.2, Figure 4.3**). The number of visiting individuals among the insect groups was not significantly different (Friedman test, $p > 0.05$). Species of *Chaloenus* was identified as *C. latifrons*. The floral scent released attracted firstly *Colocasiomyia* flies (0710). These flies were highly active, moved rapidly from the appendix to the upper pistillate flower zone and mated (appendix, staminate and pistillate flower zone). *Colocasiomyia* flies sucked on the surface of the interpistillar staminodes and inner spathe limb, fed on the liquid secreted from the stigma and damaged appendix tissues gnawed by *Chaloenus* beetles. Two *Chaloenus* beetles arrived (0730) and chewed the interpistillar staminodes and appendix. When the floral scent reduced (0900), all insects were less active and mostly remained inside the lower chamber until the following day except *Chaloenus* beetles which remained on the appendix.

At the onset of staminate anthesis, most *Colocasiomyia* flies remained on the staminate flower zone and then fed on the pollen during pollen release (0610 – 0625). Pollen was fully adhered

onto *Colocasiomyia* flies. Prior to leaving, these flies moved to the appendix to clean themselves (similar in *S. calyptrata*) and some crawled onto *Chaloenus* beetles and transferred the adhered pollen onto the bodies of *Chaloenus* beetles (0630 – 0715). *Chaloenus* beetles laid eggs on the appendix and left by 0900. This marked the end of the anthesis of *S. laxipistillata. Colocasiomyia* flies are considered as the main pollinators and *Chaloenus* beetles are considered as the opportunistic pollinator of *S. laxipistillata* as their visiting behaviours were found similar to *S. roh* Ar1240.

4.3.2 Fruit set

The percentage of natural fruit set for all investigated species of the *S. calyptrata* complex was high (80.92 – 97.08 %) except *S. pantiensis* which was slightly lower (71.24 %). Bagged fruit set (for effective pollination by smallest insects) was slightly lower than natural fruit set: *S. baangongensis* (72.11 % bagged fruit set: 93.70 % natural fruit set, $p < 0.05$, Mann-Whitney test), *S. giamensis* (70.57 %: 80.92 %, $p < 0.05$, Mann-Whitney test) and *S. roh* Ar2445 (67.38 %: 94.90 %, $p < 0.05$, Mann-Whitney test). Self-compatibility test of *S. baangongensis, S. giamensis* and *S. roh* Ar2445 did not set any fruit (**Table 4.3**).

4.3.3 Pollen Count

Among the insects, *Cycreon* beetles carried the highest number of pollen: *S. baangongensis* 1608 ± 3381 (120 – 11700 pollen, $p < 0.0006$, Kruskal-Wallis test); *S. roh* Ar1240 285 ± 545 (8 – 1260, $p < 0.045$ Kruskal-Wallis test) and *S. giamensis* 251 ± 230 (8 – 760, $p < 0.001$, Kruskal-Wallis test) (**Table 4.4**). *Parastasia* beetle is highly sporadic thus is not sampled.

Table 4.3. The mean ± standard deviation of number of pistillate flowers per inflorescence, developed fruits per infructescence, percentage of natural fruit set, developed seeds per fruit, percentage of bagged fruit set (for effective pollination by smallest insects) and self-pollination of the investigated species of the Calyptrata complex. n represents the number of replicates.

	Number of pistillate flowers per inflorescence	Developed fruits per infructescence	Natural fruit set (%)	Developed seeds on 10 fruits from 5 infructescences (n = 50).	Bagged fruit set (%)	Self-pollination (%)
S. adducta	681.80 ± 135.73 (n = 5)	560.14 ± 106.93 (n = 7)	82.16	9.12 ± 3.65	-	-
S. baangongensis	805 ± 29.11 (n = 6)	754.29 ± 85.97 (n = 7)	93.70	10.74 ± 1.36	72.11 (n = 4)	0 (n = 4)
S. caesia	679.67 ± 123.23 (n = 6)	556.40 ± 69.93 (n = 5)	81.86	19.40 ± 9.14	-	-
S. calyptrata	360.80 ± 139.12 (n = 5)	320.33 ± 179.06 (n = 6)	88.78	13.92 ± 4.50	-	-
S. giamensis	807 ± 192.83 (n = 12)	653 ± 132.68 (n = 7)	80.92	11.88 ± 4.68	76.75 (n = 5)	0 (n = 5)
S. laxipistillata	608 ± 138.94 (n = 6)	506.80 ± 35.08 (n = 5)	83.36	9.74 ± 3.48	-	-
S. muluensis	1115.80 ± 160.50 (n = 5)	1083.20 ± 196.76 (n = 5)	97.08	22.68 ± 10.13	-	-
S. pantiensis	644.80 ± 197.79 (n = 5)	459.33 ± 135.18 (n = 6)	71.24	12.78 ± 4.59	-	-
S. pseudoniahensis	886.40 ± 175.57 (n = 5)	737.60 ± 56.15 (n = 5)	82.21	13.96 ± 6.45	-	-
S. roh Ar1240	705 ± 217.54 (n = 5)	618.44 ±183.79 (n = 9)	87.72	11.88 ± 3.56	-	-
S. roh Ar2445	854.44 ± 138.26 (n = 9)	810.83 ± 139.47 (n = 12)	94.90	12.58 ± 6.50	67.38 (n = 7)	0 (n = 4)

Table 4.4. The mean ± standard deviation and range number of adhered pollen grains (*S. baangongensis*, *S. giamensis* and *S. roh* Ar1240) on insect visitors. Significant different (Mann-Whitney) between adhered pollen of each insect group are indicated with alphabets.

Insect visitors	Plant taxa		
	S. baangongensis (n = 4)	*S. giamensis* (n = 3)	*S. roh* Ar1240 (n = 3)
Atheta sp.	0 (n = 1)	0 (n = 1)	-
Chaloenus spp.	78 ± 31^{ab}	113 ± 51^{ab}	28 ± 17
	56 – 100	48 – 220	8 – 36
	(n = 2)	(n = 10)	(n = 3)
Colocasiomyia spp.	89 ± 99^{ac}	38 ± 45^{abc}	19 ± 12^{a}
	12 – 356	8 – 172	4 – 40
	(n = 12)	(n = 12)	(n = 16)
Cycreon spp.	1608 ± 3381^{abc}	251 ± 230^{ac}	285 ± 545^{a}
	120 – 11700	8 – 760	8 – 1260
	(n = 13)	(n = 8)	(n = 5)

In *S. baangongensis, Colocasiomyia* flies carried more pollen load than *Chaloenus* beetles (*Colocasiomyia* 89 ± 99, 12 – 356: *Chaloenus* 78 ± 31, 56 – 100, $p < 0.0002$, Mann Whitney test). However, *Colocasiomyia* flies carried less pollen than *Chaloenus* beetles in *S. roh* Ar1240 (*Colocasiomyia* 19 ± 12, 4 – 40: *Chaloenus* 28 ± 17, 8 – 36, $p > 0.5$ Mann Whitney test) and *S. giamensis* (*Colocasiomyia* 38 ± 45, 8 – 172: *Chaloenus* 113 ± 51, 48 – 220, $p < 0.008$, Mann Whitney test). Pollen was not found on the *Atheta* beetles. *Parastasia* beetle was not sampled as the visitation of this beetle is highly sporadic (**Table 4.4, Figure 4.14, 4.15**).

4.3.4 Pollen View

4.3.4.1 Adhered Pollen of *S. baangongensis* on the Insect Visitors

Under SEM, the shape and size of the pollen of *S. baangongensis* (**Figure 4.16 A**) was similar to the adhered pollen on *Cycreon* beetles (**Figure 4.16 B**) and *Colocasiomyia* flies (**Figure 4.16 H**). *Cycreon* beetles have more body parts (abdomen, elyptra, femur, pronotum, tarsus and tibia, **Figure 4.16 C, D, E**) covered with adhered pollen than *Colocasiomyia* flies (tarsus, **Figure 4.16 G**).

4.3.4.2 Adhered Pollen of *S. giamensis* on the Insect Visitors

Under SEM, the shape and size of the pollen of *S. giamensis* (**Figure 4.17 A**) was similar to the adhered pollen on *Cycreon* beetles (**Figure 4.17 B**) and *Colocasiomyia* flies (**Figure 4.17 H**). *Cycreon* beetles have more body parts (abdomen, femur, pronotum and tibia, **Figure 4.17 C, D, E**) observed with adhered pollen than *Colocasiomyia* flies (tarsus, **Figure 4.17 F & G**).

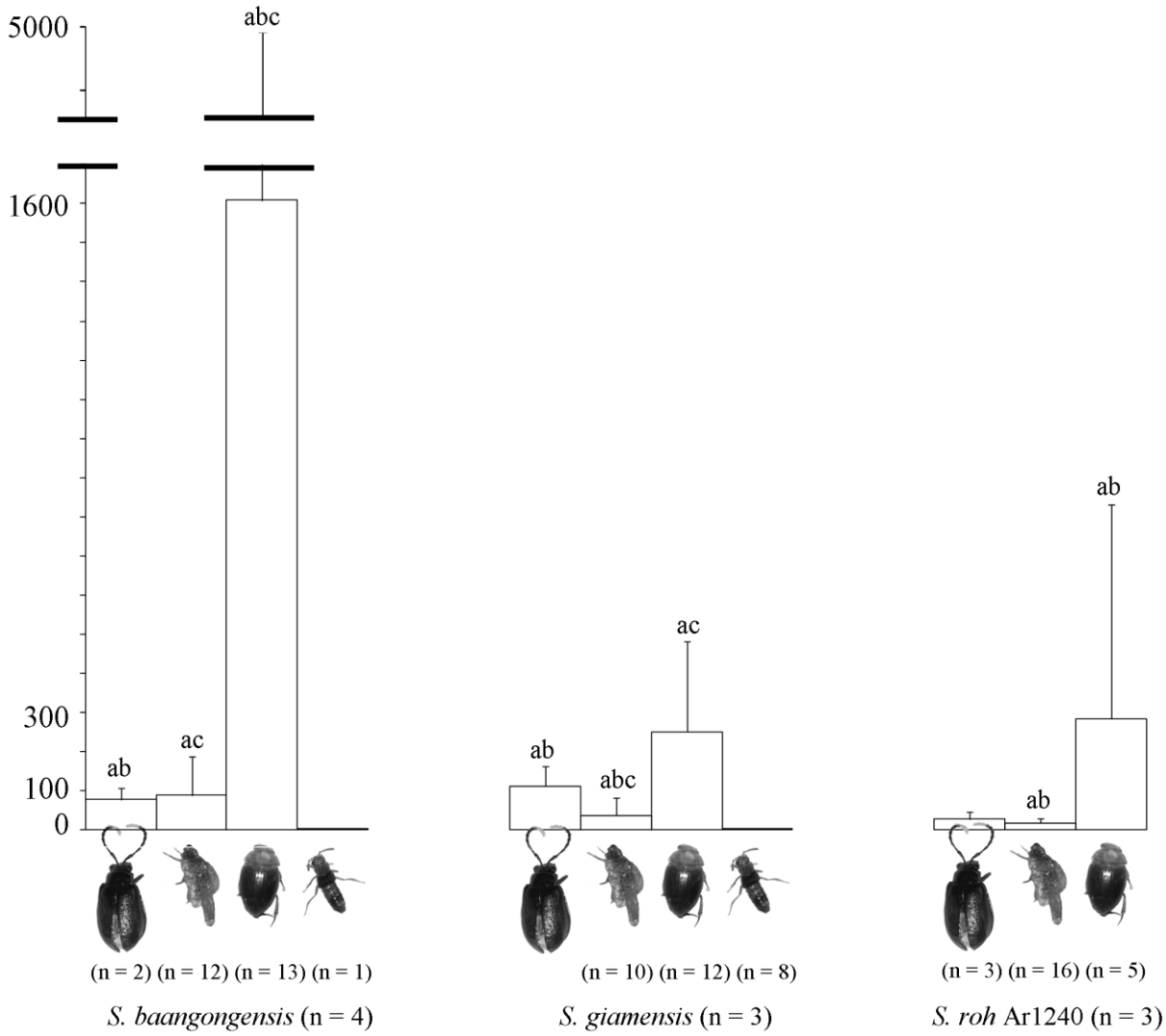

Figure 4.14: The mean ± standard deviation and range number of adhered pollen grains (*S. baangongensis*, *S. giamensis* and *S. roh* Ar1240) on insect visitors. Significant different (Mann-Whitney) between adhered pollen of each insect group are indicated with different alphabets.

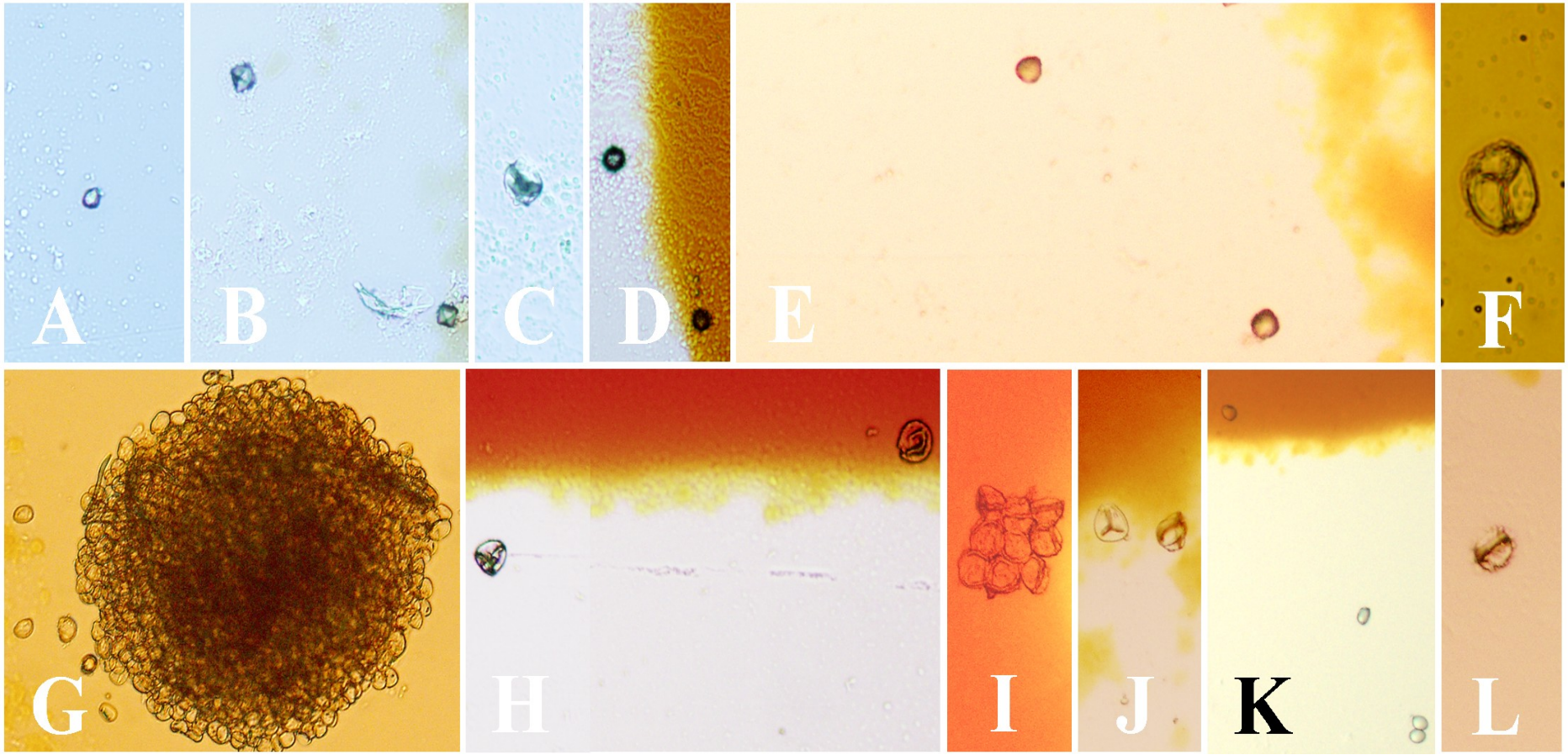

Figure 4.15. Pollen view under the compound microscope. A – F. *Schismatoglottis giamensis*: (A – C) other unidentified pollen adhered on *Chaloenus* beetles (200x magnification); (D) host pollen on *Chaloenus* beetles (200x magnification); (E) host pollen on *Cycreon* beetles (200x magnification); (F) unidentified pollen on *Cycreon* beetles (400x magnification). G – L. *Schismatoglottis baangongensis*: (G) clumps of host pollen on *Cycreon* beetles (200x magnification); (H – J) unidentified pollen on *Cycreon* beetles (200x magnification); host pollen (all granules) (K) (100x magnification) and unidentified pollen (L) on *Colocasiomyia* flies (200x magnification).

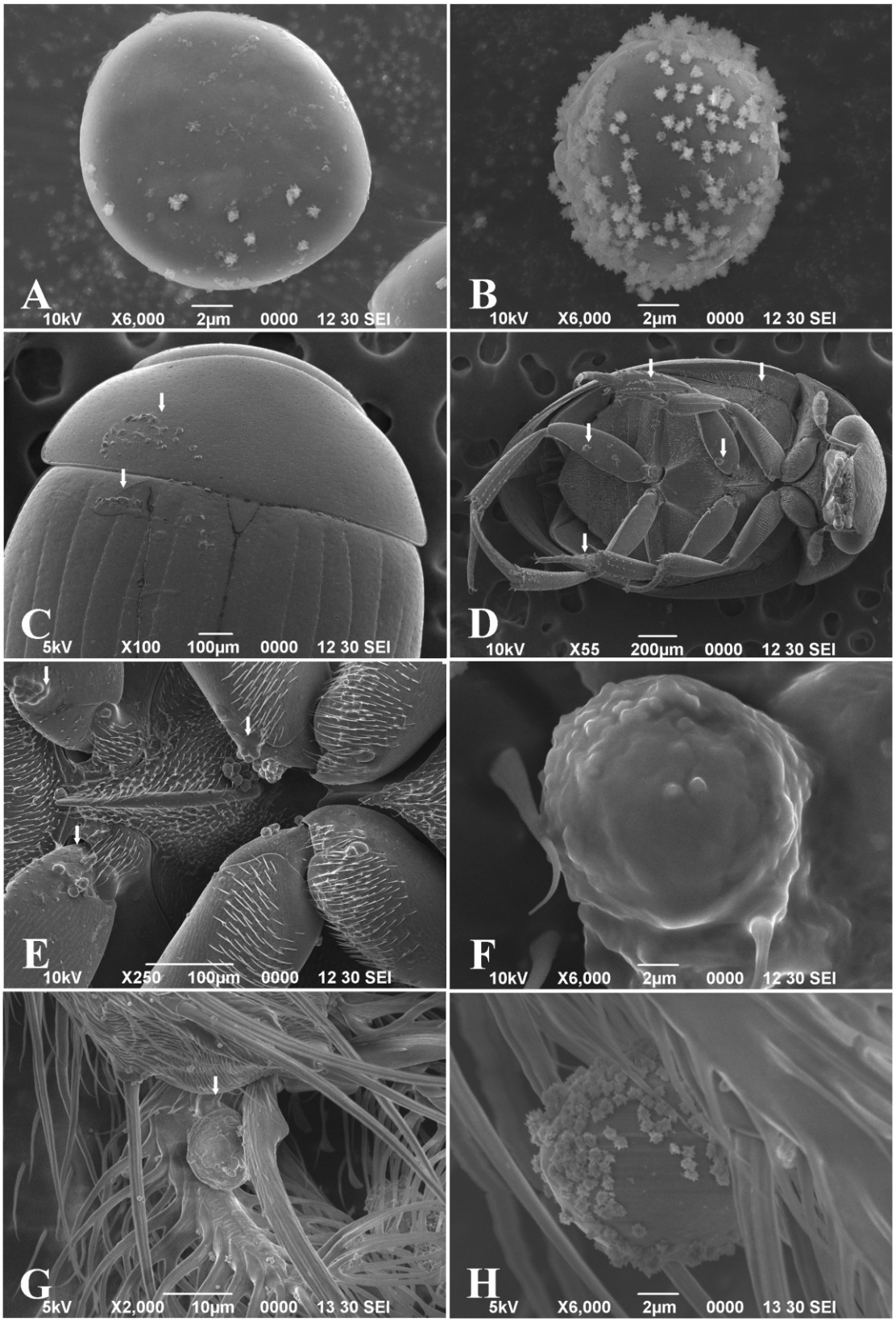

Figure 4.16. Adhered pollen of *S. baangongensis* on visited insects. A. Host pollen grain; B. Adhered host pollen on *Cycreon* beetles; C – E. Adhered host pollen on tibia, femur, pronotum and abdomen of *Cycreon* beetles; F – H. Adhered host pollen on tibia of *Colocasiomyia* flies.

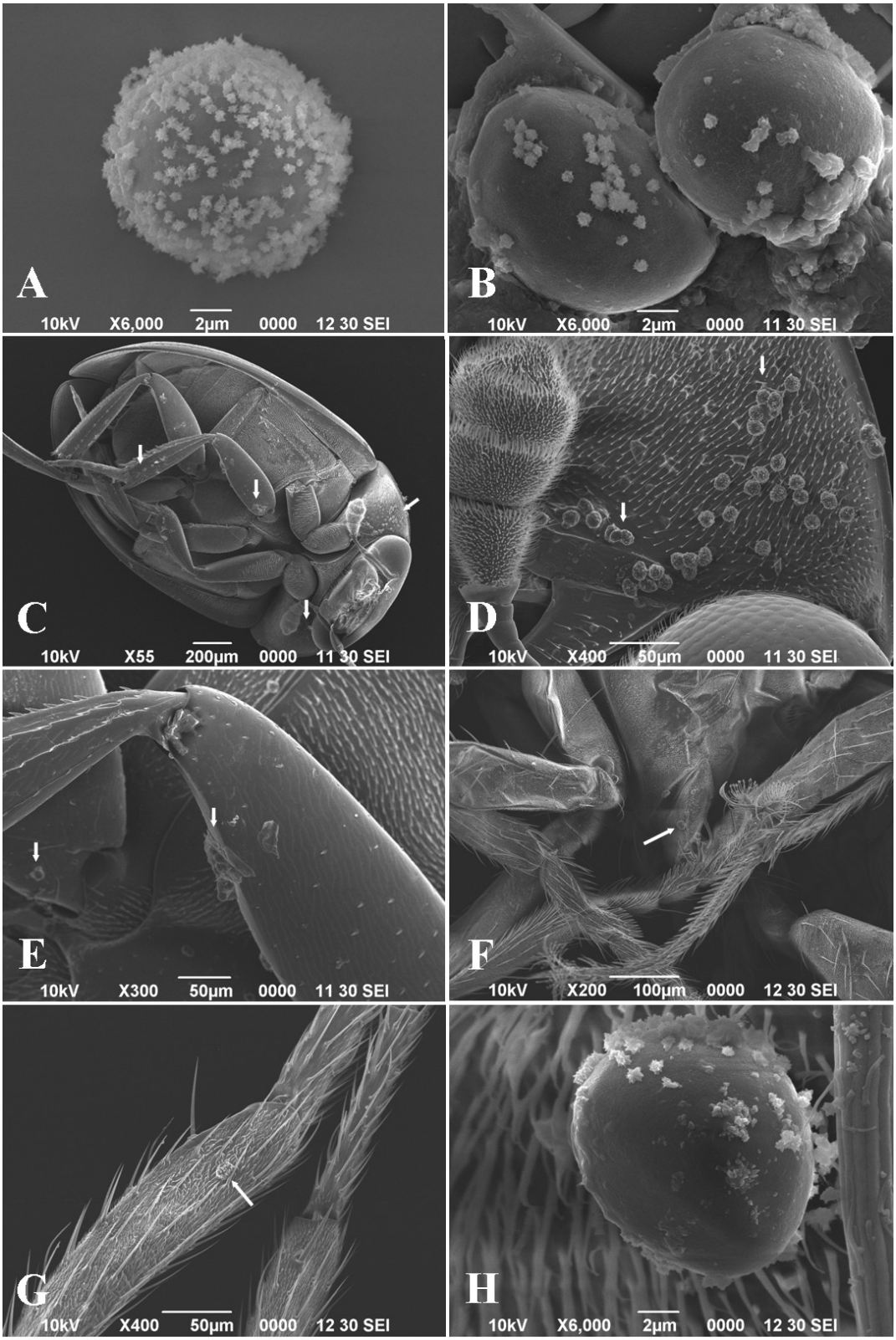

Figure 4.17. Adhered pollen of *S. giamensis* on visited insects. A. Host pollen grain; B. Adhered host pollen on *Cycreon* beetles; C. Adhered host pollen on pronotum and elyptra of *Cycreon* beetles; D & E. Adhered host pollen on tarsus, tibia, femur and abdomen of *Cycreon* beetles; F. Adhered host pollen on abdomen of *Cycreon* beetle.; G & H. Adhered host pollen on tarsus of *Colocasiomyia* flies.

4.3.5 Breeding Test

4.3.5.1 Larvae Development on the Spathe Limb of *S. roh* Ar2445

Five days after the pistillate anthesis, ca three larvae (ca 0.5 mm diam.) were observed on the spathe limb of *S. roh* Ar2445. By day 12, only one *Colocasiomyia* fly emerged. One individual large larva was observed (day 7), turned pupa (ca 3.5 mm long x ca 1.5 mm wide) (day 9) but later remained unhatched (**Figure 4.18 F, G**). Unidentified whitish or brownish acari mites were also observed (**Figure 4.18 E**).

4.3.5.2 Larvae Development on the Pistillate Flower Zone of *S. roh* Ar2445

Four days after the pistillate anthesis, ca five larvae (ca 1.5 mm long) were observed (**Figure 4.18 C, D**). By day 15, 17, 18, 19, 22 and 24, the number of emerged *Colocasiomyia* flies were 1, 2, 5, 4, 1 and 1 respectively. Unidentified whitish or brownish beetles were also observed.

4.3.5.3 Larvae Development on the Pistillate Flower Zone of *S. giamensis*

Five days after the pistillate anthesis, ca five larvae were observed. By day 12, 13 and 17, ca 2, 3 and 4 *Colocasiomyia* flies emerged respectively. However, some pupae and adults of *Colocasiomyia* flies could not escaped from the degraded and sticky lower spathe (**Figure 4.18 A**).

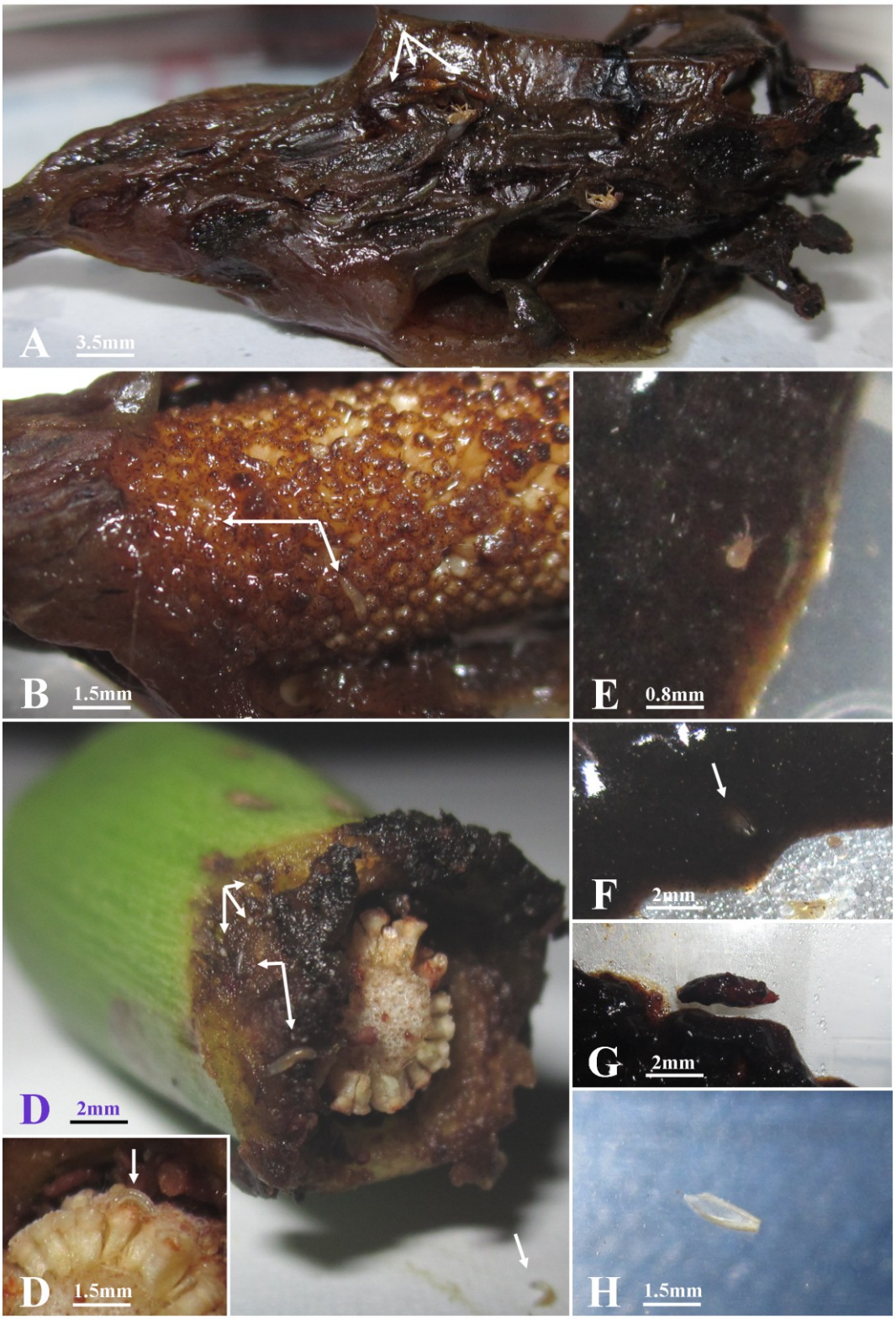

Figure 4.18. Breeding test. **A – B.** Pistillate flower zone of *S. giamensis*: (A) Two *Colocasiomyia* flies and four pupae (white arrows) cannot emerge as they covered by degraded lower spathe; (B) Larvae (day 5). **C – H.** *Schismatoglottis roh* Ar2445**:** (C – E) Larvae and unidentified white brownish beetle (day 5); (F) an individual large larva remain inside the degrading spathe (day 7) and (G) it turned into pupa (day 9); (H) Cocoon of the escaped *Colocasiomyia* fly.

4.3.6 Thermogenesis

4.3.6.1 Thermogenesis of *S. adducta*

The appendix and staminate flower zone of *S. adducta* is biphasic. The first peak occured during pistillate anthesis, the second was during staminate anthesis (**Figure 4.19**).

The heat production during pistillate anthesis occured between 0200 – 0900. The temperature of the appendix remained the highest, subsequently staminate flower zone, pistillate flower zone and ambient temperature. The appendix reached to maximum 34.43 °C ± 1.13 (9.41 °C ± 1.22 above the ambient) (0640), staminate flower zone reached 27.98 °C ± 0.41 (2.94 °C ± 0.24 above the ambient) (0645) and pistillate flower zone reached 25.40 °C ± 0.08 (0.36 °C ± 0.08 above the ambient) (0645).

During inter-anthesis, no thermogenesis was detected. Between 0900 – 2200, the ambient temperature remained the highest (up to 29.67 °C), followed by appendix (up to 28.94 °C), staminate flower zone (up to 28.92 °C) and pistillate flower zone (up to 28.77 °C) (except between 1120 – 1800, the temperature of the staminate flower zone (up to 30.59 °C) remained higher than the appendix (up to 30.45 °C). Between 2200 – 0245 (day 2), only the appendix (up to 27.34 °C) and staminate flower zone (up to 27.29 °C) remained higher than ambient temperature (up to 27.19 °C).

Between 0245 – 0730, the heat production of staminate anthesis began. The temperature of the appendix remained the highest, subsequently staminate flower zone and pistillate flower zone. The appendix reached to maximum 30.23 °C ± 1.54 (4.76 °C ± 2.27 above the ambient)

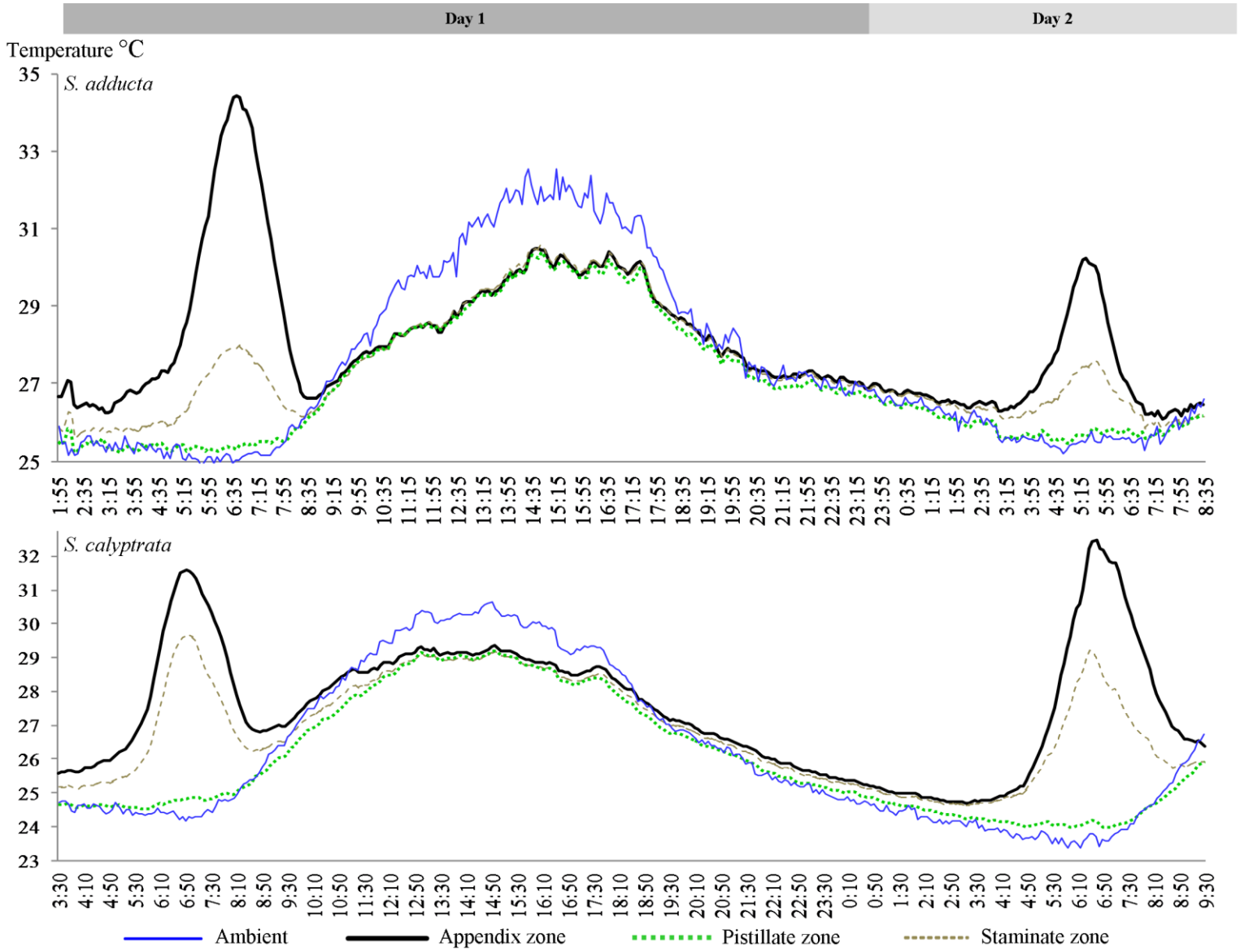

Figure 4.19: Thermogenesis of *S. adducta* and *S. calyptrata.*

(0525); staminate flower zone reached 27.59 ℃ ± 0.03 (2.05 ℃ ± 1.07 above the ambient) (0540) and pistillate flower zone reached 25.84 ℃ ± 0.60 (0.30 ℃ ± 3.25 above the ambient) (0540). At the end of the staminate anthesis (after 0730), the thermogenesis of *S. adducta* ended with the ambient temperature remined higher than the appendix, staminate flower zone and pistillate flower zone.

4.3.6.2 Thermogenesis of *S. calyptrata*

The appendix and staminate flower zone of *S. calyptrata* is biphasic, with the first peak occured during pistillate anthesis, the second was during staminate anthesis. The pistillate flower zone increase slightly (< 0.67 ℃) above the ambient temperature in both anthesis (**Figure 4.19**).

The heat production during pistillate anthesis occured between 0330 – 1100. The temperature of the appendix remained the highest, subsequently staminate flower zone, pistillate flower zone and ambient temperature. The appendix reached to maximum 31.58 ℃ ± 3.59 (7.41 ℃ ± 3.29 above the ambient) (0650), staminate flower zone reached 29.65 ℃ ± 2.33 (5.38 ℃ ± 2.01 above the ambient) (0655) and pistillate flower zone reached 24.85 ℃ ± 0.61 (0.60 ℃ ± 0.32 above the ambient) (0700).

Between 1100 – 1930, no thermogenesis was detected. The ambient temperature remained the highest (up to 30.67 °C), followed by appendix (up to 29.28 °C), staminate flower zone (up to 29.10 °C) and pistillate flower zone (up to 29.06 °C), except between 1300 – 1540, the temperature of the pistillate flower zone (up to 29.20 °C) remained higher than the staminate flower zone (up to 29.17 °C).

Between 2130 – 0920 (day 2), the heat production of staminate anthesis began. The temperature of the appendix remained the highest, subsequently staminate flower zone and pistillate flower zone. The appendix reached to maximum 32.47 °C ± 4.62 (8.78 °C ± 5.02 above the ambient) (0640); staminate flower zone reached 28.70 °C ± 2.19 (4.94 °C ± 2.73 above the ambient) (0630) and pistillate flower zone reached 24.13 °C ± 0.69 (0.34 °C ± 0.57 above the ambient) (0635). At the end of the staminate anthesis (after 0920), the thermogenesis of *S. calyptrata* ended with the ambient temperature remined higher than the appendix, staminate flower zone and pistillate flower zone.

4.3.6.3 Thermogenesis of *S. giamensis*

The appendix and staminate flower zone of *S. giamensis* is biphasic. The first peak occured during pistillate anthesis, the second was during staminate anthesis (**Figure 4.20**).

The heat production during pistillate anthesis occured between 0200 – 1025, with the temperature of the appendix remained the highest, subsequently staminate flower zone, pistillate flower zone and ambient temperature. The appendix reached to maximum 33.50 °C ± 1.41 (10.34 °C ± 1.87 above the ambient) (0700), staminate flower zone reached 26.77 °C ± 0.36 (3.60 °C ± 0.81 above the ambient) (0700) and pistillate flower zone reached 23.68 °C ± 0.19 (0.45 °C ± 0.56 above the ambient) (0630).

During inter-anthesis, no thermogenesis was detected. Between 1025 – 1715, the ambient temperature remained the highest (up to 30.47 °C), followed by staminate flower zone (up to 29.06 °C), pistillate flower zone (up to 28.96 °C) and appendix (up to 28.64 °C). Between 1715 – 0145 (day 2), only staminate flower zone (up to 28.31 °C) and pistillate flower zone

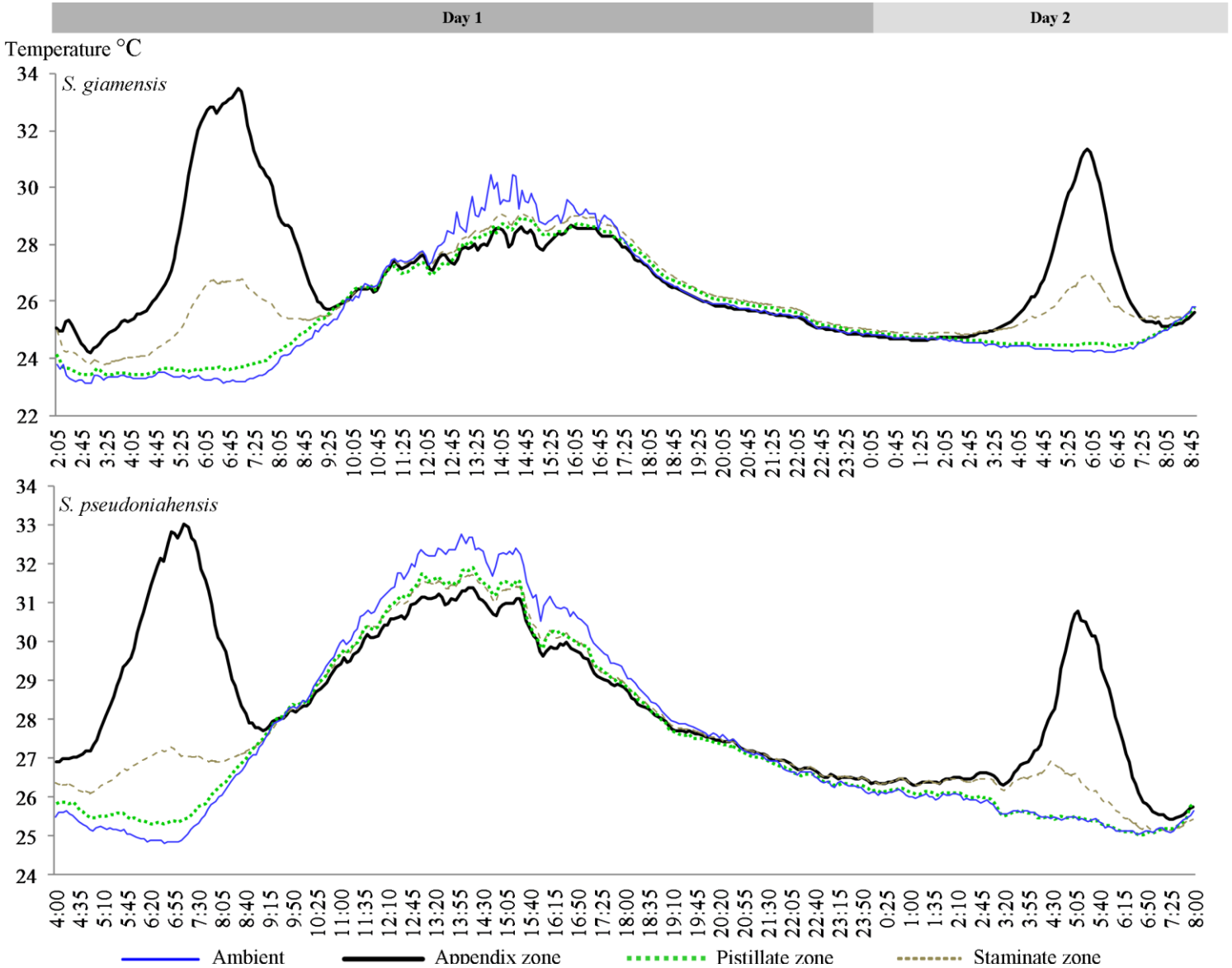

Figure 4.20: Thermogenesis of *S. giamensis* and *S. pseudoniahensis*

(up to 28.09 °C) remained higher than ambient temperature (up to 28.03 °C).

Between 0145 – 0850, the heat production of the staminate anthesis began. The temperature of the appendix remained the highest, subsequently staminate flower zone, pistillate flower zone and ambient temperature. The appendix reached at a maximum of 31.37 °C ± 0.35 (7.11 °C ± 0.69 above the ambient) (0640), staminate flower zone reached 26.94 °C ± 1.73 (2.70 °C ± 0.69 above the ambient) (0645) and the pistillate flower zone reached 24.52 °C ± 1.17 (0.24 °C ± 0.15 above the ambient) (0645). At the end of the staminate anthesis (after 0850), the thermogenesis of *S. giamensis* ended with the ambient temperature remined higher than the appendix, staminate flower zone and pistillate flower zone.

4.3.6.4 Thermogenesis of *S. pseudoniahensis*

The appendix and staminate flower zone of *S. pseudoniahensis* is biphasic. The first peak occured during pistillate anthesis, the second was during staminate anthesis (**Figure 4.20**).

The heat production during pistillate anthesis occured between 0400 – 1000. The temperature of the appendix remained the highest, subsequently staminate flower zone, pistillate flower zone and ambient temperature. The appendix reached to maximum 33.04 °C ± 1.22 (8.06 °C ± 1.26 above the ambient) (0710), staminate flower zone reached 27.30 °C ± 0.22 (2.43 °C ± 0.70 above the ambient) (0650) and pistillate flower zone reached 25.61 °C ± 0.70 (0.43 °C ± 0.18 above the ambient) (0540).

During inter-anthesis, no thermogenesis was detected. Between 1000 – 1900, the ambient temperature remained the highest (up to 32.70 °C), followed by pistillate flower zone (up to

31.90 °C), staminate flower zone (up to 31.74 °C) and appendix (up to 31.41 °C). Within 1900 – 2100, only appendix (up to 27.94 °C) was found remained higher than pistillate flower zone (up to 27.91°C).

Between 2100 – 0800, the heat production of staminate anthesis began. The temperature of the appendix remained the highest, subsequently staminate flower zone, pistillate flower zone and ambient temperature. The appendix reached to maximum 30.78 °C ± 1.68 (5.35 °C ± 1.71 above the ambient) (0510), staminate flower zone reached 26.92 °C ± 0.21 (1.45 °C ± 0.29 above the ambient) (0430) and pistillate flower zone reached 25.50 °C ± 0.16 (0.07 °C ± 0.09 above the ambient) (0510). At the end of the staminate anthesis (after 0800), the thermogenesis of *S. pseudoniahensis* ended with the ambient temperature remined higher than the appendix, staminate flower zone and pistillate flower zone.

4.3.6.5 Thermogenesis of *S. roh* Ar1240

The appendix, staminate and pistillate flower zone of *S. roh* Ar1240 is biphasic. The first peak occured during pistillate anthesis, the second was during staminate anthesis (**Figure 4.21**).

The heat production during pistillate anthesis occured between 0330 – 0940. The temperature of the appendix remained the highest, subsequently staminate flower zone, pistillate flower zone and ambient temperature. The appendix reached to maximum 30.05 °C ± 3.64 (5.29 °C ± 1.99 above the ambient) (0645), staminate flower zone reached 28.34 °C ± 2.28 (3.53 °C ± 0.71 above the ambient) (0630) and pistillate flower zone reached 25.67 °C ± 1.72 (0.86 °C ± 0.14 above the ambient) (0630).

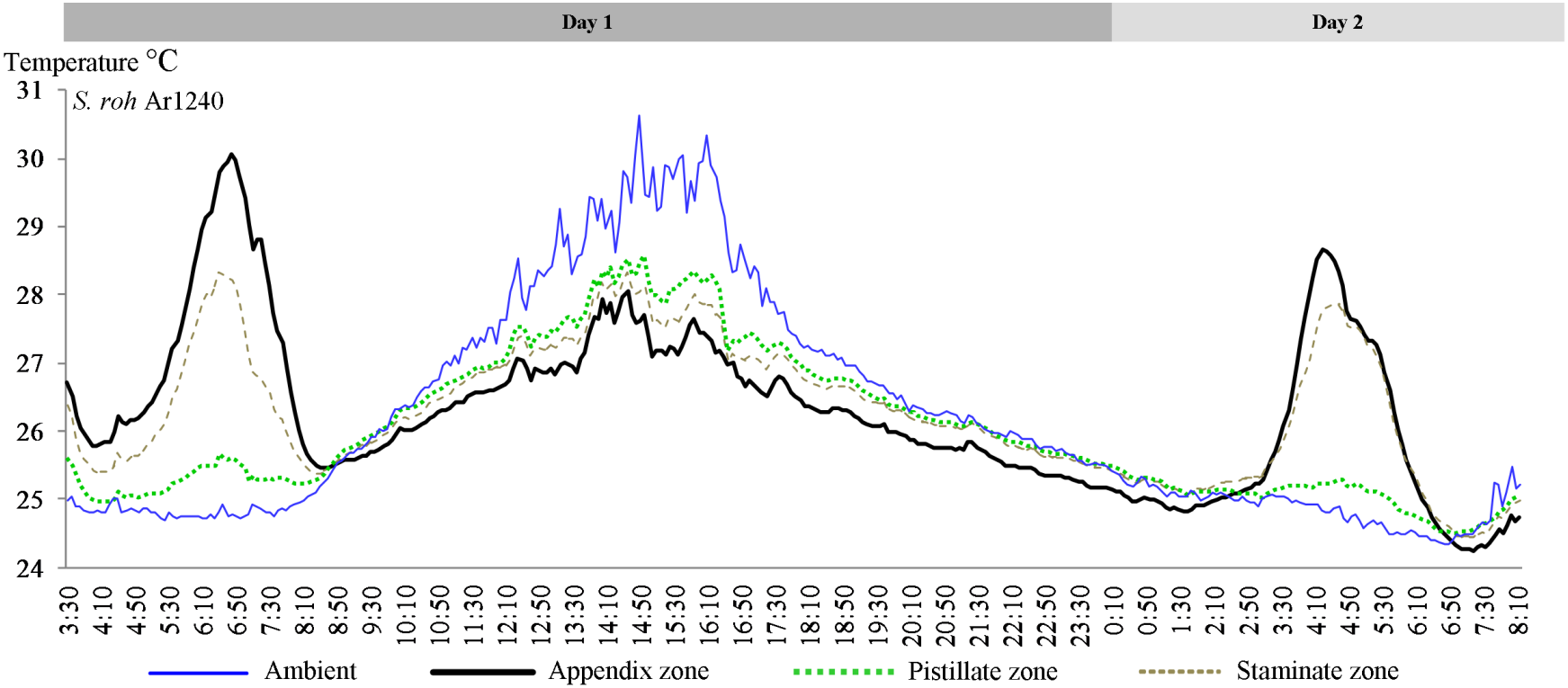

Figure 4.21: Thermogenesis of *S. roh* Ar1240.

During inter-anthesis, no thermogenesis was detected. Between 0940 – midnight, the ambient temperature remained the highest (up to 30.62 °C), followed by pistillate flower zone (up to 28.56 °C), staminate flower zone (up to 28.34 °C) and appendix (up to 28.05 °C). At midnight until 0230 (day 2), only staminate flower zone (up to 25.42 °C) and pistillate flower zone (up to 25.48°C) remained higher than ambient temperature (up to 25.41 °C).

Between 0230 – 0730, the heat production of staminate anthesis began. The temperature of the appendix remained the highest, subsequently staminate flower zone, pistillate flower zone and ambient temperature. The appendix reached to maximum 28.67 °C ± 3.94 (3.83 °C ± 4.50 above the ambient) (0420), staminate flower zone reached 27.87 °C ± 2.09 (3.01 °C ± 2.60 above the ambient) (0435) and pistillate flower zone reached 25.28 °C ± 0.40 (0.55 °C ± 0.21 above the ambient) (0445). At the end of the staminate anthesis (after 0730), the thermogenesis of *S. roh* Ar1240 ended with the ambient temperature remined higher than the appendix, staminate flower zone and pistillate flower zone.

4.3.7 Floral VOCs Analyses

4.3.7.1 Floral VOCs of Inflorescences Analyzed by BPX-5 Intermediate Polar Column

A total of four VOCs were identified in inflorescences of six species of Calyptrata complex (*S. roh* Ar1240*, S. roh* Ar2445*, S. giamensis, S. baangongensis, S. adducta, S. muluensis* and *S. calyptrata*) (**Table 4.5**). All identified volatile compounds belong to ester compound class. All the six species of Calyptrata complex emitted high relative amounts (> 94 %) of 3-butenoic acid, 3-methyl-, methyl ester and small amount of 2-butenoic acid, 3-methyl-, methyl ester (0.05 – 0.85 %). Methyl benzoate (1.32 – 5.04 %) and 3-buten-1-ol, 3-methyl- (< 0.03 %)

Table 4.5. Chemical composition (mean relative amount of each VOC) of the floral scent of six species of Calyptrata complex analyzed by BPX-5 intermediate polar column in splitless mode. Samples were obtained by dynamic headspace during the interval of highest perceivable scent emission in the course of the pistillate (♀) and staminate (♂) phases of anthesis. *Sadduc* (*Schismatoglottis adducta*), *Sbaan* (*Schismatoglottis baangongensis*), *Scalyp* (*Schismatoglottis calyptrata*), *Sroh* Ar2445 (*Schismatoglottis roh* Ar2445), *Sgiam* (*Schismatoglottis giamensis*), *Smulu* (*Schismatoglottis muluensis*), *Sroh* Ar1240 (*Schismatoglottis roh* Ar1240). The compounds are listed according to Kovats retention index (RI).

Odour compounds	Retention time	Retention index	*Sadduc* ♀ (n=3)	*Sbaan* ♀ (n=6)	*Scalyp* ♀ (n=5)	*Sgiam* ♀ (n=3)	*Smulu* ♀ (n=2)	*Sroh* Ar1240 ♀(n=1)	*Sroh* Ar2445 ♀(n=2)	*Sbaan* ♂ (n=1)	*Scalyp* ♂ (n=2)	*Smulu* ♂ (n=2)
3-butenoic acid, 3-methyl-, methyl ester	6.90	800	98.48	94.80	99.95	99.26	97.47	99.85	99.95	0	0	0
2-butenoic acid, 3-methyl-, methyl ester	8.69	845	0.17	0.15	0.05	0.74	0.85	0.15	0.05	0	0	0
3-buten-1-ol, 3-methyl-	11.80	922	0.03	0.03	0	0	0.01	0	0	0	0	0
Methyl benzoate	18.20	1270	1.32	5.04	0	0	1.67	0	0	0	0	0

were only present in *S. baangongensis, S. muluensis* and *S. adducta.* The floral scent patterns among all species were not significantly different (one-way PERMANOVA, $p > 0.15$). However, the pairwise comparisons was significant different between *S. calyptrata – S. giamensis* (Bonferroni significance, $p < 0.016$), *S. calyptrata – S. muluensis* (Bonferroni significance, $p < 0.048$), *S. calyptrata – S. baangongensis* (Bonferroni significance, $p < 0.015$) and *S. calyptrata – S. adducta* (Bonferroni significance, $p < 0.02$). The floral scent patterns did not have interaction effect with any of the three factors (two-way PERMANOVA, $p > 0.24$), and there was no significant interaction effect among the three factors (two-way PERMANOVA, $p > 0.99$). No compound was detected to be present in inflorescences trapped during staminate anthesis of *S. baangongensis, S. calyptrata* and *S. muluensis.*

4.3.7.2 Floral VOCs of Inflorescences Analyzed by BP20 Polar Column

A total of nine VOCs were identified in inflorescences of nine Calyptrata species (excluding *S. pseudoniahensis*) (**Table 4.6**). The four identified volatile compounds belong to ester compound class. The mean relative amount among the floral VOCs for intact inflorescence was significantly different in all investigated species of Calyptrata complex (Friedman test, $p < 0.003$). All Calyptrata species emitted high relative amounts (96.35 – 99.89 %) of 3-butenoic acid, 3-methyl-, methyl ester and small relative amount of 2-butenoic acid, 3-methyl-, methyl ester (0.09 – 0.72 %). 3-buten-1-ol, 3-methyl- (0.02 – 0.32 %) was only absent in *S. caesia*. A small amount (0.02 – 0.79 %) of unidentified compounds were only found in *S. giamensis* (unidentified compound 2 and 3), *S laxipistillata* (unidentified compound 5) and *S. roh* Ar1240 (unidentified compound 1 and 4). Species belonging to the Calyptrata complex emitted similar VOCs composition were: *S. baangongensis, S. muluensis, S. pantiensis* and *S. adducta* (four identified compounds; 3-butenoic acid, 3-methyl-, methyl ester; 2-butenoic acid,

Table 4.6. Chemical composition (mean relative amount of each VOC) of the floral scent of ten species of Calyptrata complex analyzed by BP20 polar column in split mode. Samples were obtained by dynamic headspace during the interval of highest perceivable scent emission in the course of the pistillate phases of anthesis. *Sadduc* (*Schismatoglottis adducta*), *Sbaan* (*Schismatoglottis baangongensis*), *Scae* (*Schismatoglottis caesia*), *Scalyp* (*Schismatoglottis calyptrata*), *Sgiam* (*Schismatoglottis giamensis*), *Slaxi* (*Schismatoglottis laxipistillata*), *Smulu* (*Schismatoglottis muluensis*), *Spanti* (*Schismatoglottis pantiensis*), *Sroh* Ar1240 (*Schismatoglottis roh* Ar1240) and *Sroh* Ar2445 (*Schismatoglottis roh* Ar2445). Significant different between each Calyptrata species are indicated with different alphabets in one-way PERMANOVA. The compounds are listed according to Kovats retention index (RI).

Odour compounds	Retention time	Retention index	*Sadduc* abcdeghi (n=6)	*Sbaan* cdefhi (n=6)	*Scae* bcefh (n=6)	*Scalyp* abcdeghi (n=6)	*Sgiam* ef (n=5)	*Slaxi* acefhi (n=6)	*Smulu* abcdeghi (n=6)	*Spanti* abcdefhi (n=4)	*Sroh* Ar1240 eghi (n=4)	*Sroh* Ar2445 abcdefghi (n=6)
3-butenoic acid, 3-methyl-, methyl ester	5.635	1119	97.97	99.44	99.54	99.89	96.35	99.06	98.37	96.63	99.43	99.76
unidentified 1	6.1	1134	0	0	0	0	0	0	0	0	0.02	0
2-butenoic acid, 3-methyl-, methyl ester	6.965	1162	0.26	0.18	0.20	0.09	0.27	0.17	0.72	0.20	0.20	0.10
unidentified 2	7.365	1075	0	0	0	0	0.05	0	0	0	0	0
unidentified 3	7.525	1079	0	0	0	0	0.79	0	0	0	0	0
3-buten-1-ol, 3-methyl-	9.410	1241	0.31	0.32	0	0.02	0.30	0.24	0.12	0.04	0.28	0.14
unidentified 4	9.761	1252	0	0	0	0	0	0	0	0	0.02	0
Methyl benzoate	19.51	1608	1.46	0.07	0.26	0	3.03	0.44	0.75	3.13	0.05	0
unidentified 5	16.395	1484	0	0	0	0	0	0.10	0	0	0	0

3-methyl-, methyl ester; 3-buten-1-ol, 3-methyl- and Methyl benzoate); *S. calyptrata* and *S. roh* Ar2445 (three identified compounds: 3-butenoic acid, 3-methyl-, methyl ester; 2-butenoic acid,3-methyl-, methyl ester; 3-buten-1-ol, 3-methyl-). The floral scent patterns among all the species were not significantly different (one-way PERMANOVA, $F = 1.51$, $p = 0.09$), the significantly different of the pairwise comparisons between the species were listed in **Table 4.6**. The floral scent patterns have weak interaction effect (two-way PERMANOVA, $F = 1.42$, $p < 0.047$) with the factor of opportunist pollinator, but have no interaction effect (two-way PERMANOVA, $p > 0.63$) with the other two factors. There was no significant interaction effect among the three factors (two-way PERMANOVA, $p > 0.17$). The NMDS interpretation (**Figure 4.22**) showed a good ordination with the stress values = 0.02. The floral scent profile of *S. pantiensis* did not overlapped with other species in the Calyptrata complex. Individual floral scent profile of *S. baangongensis, S. calyptrata, S. roh* Ar2445*, S. caesia, S. muluensis* and *S. roh* Ar1240 were closely spaced respectively.

4.3.7.3 Floral VOCs of Different Inflorescences Parts Analyzed by BP20 Polar Column

A total of six VOCs were identified in different inflorescence parts (appendix, pistillate flower zone, spathe and staminate flower zone) of *S. baangongensis, S. giamensis* and *S. adducta* (**Table 4.7**) at both time periods (I & II). The identified volatile compounds belong to ester (4) and nitrogen containing (1) compound classes. At both periods, all different inflorescence parts of the three Calyptrata species emitted high relative amount of 3-butenoic acid, 3-methyl-, methyl ester (53.2 – 100 %), and small to moderate (0.5 – 37.6 %) Methyl benzoate (except it was high (46.8 %) in staminate flower zone of *S. baangongensis* at period

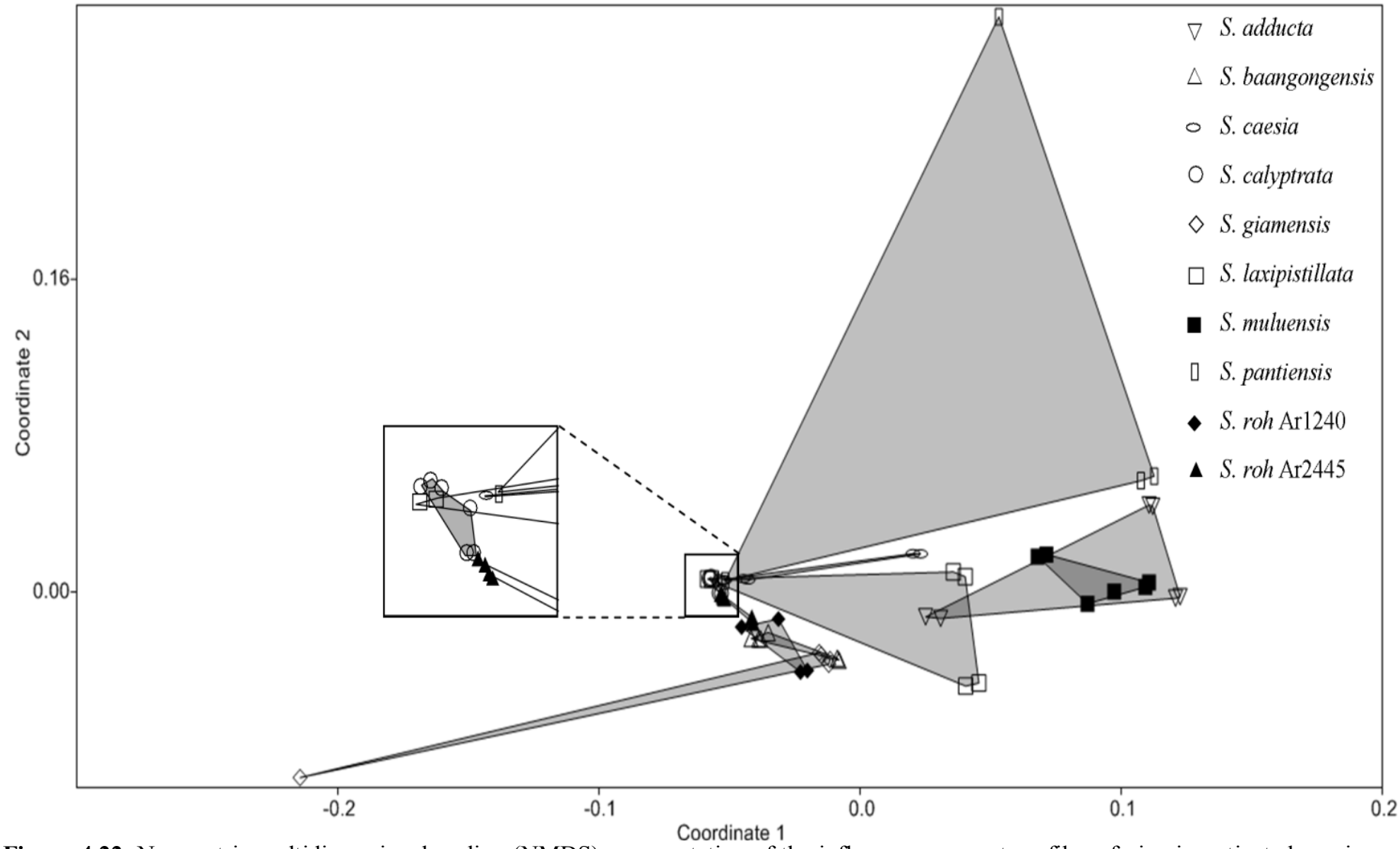

Figure 4.22. Non-metric multidimensional scaling (NMDS) representation of the inflorescence scent profiles of nine investigated species of the Calyptrata complex (stress value = 0.02).

Table 4.7. Chemical composition (relative amount (%) and amount (ng/hr) of each VOC) of the floral scent for appendix (app), pistillate flower zone (pis), spathe (spa) and staminate flower zone (sta) of *Schismatoglottis baangongensis* (*Sbaan*), *Schismatoglottis giamensis* (*Sgiam*) and *Schismatoglottis adducta* (*Sadduc*) that analyzed by BP20 polar column in split mode. Samples were obtained by dynamic headspace during time period I (0600 – 0800) and II (0815 – 1015) in the course of the pistillate phases of anthesis.

species	Odour compounds	3-butenoic acid, 3-methyl-, methyl ester	2-butenoic acid, 3-methyl-, methyl ester	Unidentified ester compound 1	3-buten-1-ol, 3-methyl-	methyl benzoate	Indole
	Retention time	5.635	6.965	7.620	9.410	19.510	35.750
	Retention index	1119	1162	1183	1241	1608	2422
Sadduc	app ^{I} (n=3)	85.2 (1925.2)	0.2 (3.9)	0.1 (3)	0.03 (0.7)	14.5 (327.2)	-
	spa ^{I} (n=3)	77.5 (441.8)	-	-	0.1 (0.7)	5.3 (30)	17.1 (97.6)
	sta ^{I} (n=4)	71.7 (196.1)	-	-	-	28.3 (77.4)	-
	pis ^{I} (n=3)	97.8 (65)	-	-	-	2.2 (1.4)	-
	app ^{II} (n=3)	100 (352.2)	-	-	-	-	-
	spa ^{II} (n=3)	62 (240.7)	-	-	0.7 (2.6)	18 (69.8)	19.3 (74.9)
	sta ^{II} (n=2)	88.2 (195)	-	-	-	11.8 (26.2)	-
	pis ^{II} (n=2)	98.3 (293.6)	-	-	-	1.7 (5)	-
Sbaan	app ^{I} (n=2)	77.6 (2120.7)	0.1 (3.6)	-	0.1 (3.9)	22.1 (605.4)	-
	spa ^{I} (n=3)	61 (1060.7)	0.1 (1)	-	0.1 (1.9)	29.3 (509.8)	9.5 (165)
	sta ^{I} (n=3)	53.2 (595)	-	-	-	46.8 (524.4)	-
	pis ^{I} (n=4)	94 (133.8)	-	-	-	6 (8.6)	-
	app ^{II} (n=2)	87.9 (2090.6)	0.2 (3.8)	-	0.1 (2.3)	11.8 (280.6)	-
	spa ^{II} (n=2)	67.7 (282.4)	-	-	8.2 (34.3)	10 (41.7)	14.1 (58.8)
	sta ^{II} (n=4)	95 (548.2)	-	-	-	5 (29.2)	-
	pis ^{II} (n=2)	99.2 (503)	-	-	-	0.8 (4.3)	-
Sgiam	app ^{I} (n=3)	96.7 (933.3)	0.1 (1.2)	-	0.1 (0.9)	3 (29.4)	-
	spa ^{I} (n=4)	95.4 (708.5)	-	-	-	4.6 (34.4)	-
	sta ^{I} (n=2)	91.5 (605.5)	-	-	-	8.5 (56)	-
	pis ^{I} (n=3)	97.5 (106.8)	-	-	-	2.5 (2.7)	-
	app ^{II} (n=3)	96.9 (479.1)	-	-	-	3.1 (15.3)	-
	spa ^{II} (n=2)	83.8 (154.9)	-	-	5.9 (10.9)	10.2 (18.9)	-
	sta ^{II} (n=2)	62.4 (177.9)	-	-	-	37.6 (107.3)	-
	pis ^{II} (n=2)	99.5 (160.6)	-	-	-	0.5 (0.8)	-

I and absent in appendix of *S. adducta* at period II). Indole (9.5 – 19.3 %) presented in the spathe of *S. baangongensis* and *S. adducta.* Small amount of unidentified ester compound 1 (0.1 %) only presented in appendix of *S. adducta* (period I). Both indole and unidentified ester compound 1 were not present in the intact inflorescence.

Among the three Calyptrata species, the appendix emitted the highest total amount (ng/hr) of floral scent at period I, following by the spathe, the staminate flower zone and the pistillate flower zone (**Table 4.7, Figure 4.23**). At period II, the total amount (ng/hr) of floral scent is varying among the three Calyptrata species: *S. baangongensis* (appendix> staminate flower zone > pistillate flower zone > spathe), *S. giamensis* (appendix > staminate flower zone > spathe > pistillate flower zone) and *S. adducta* (spathe > appendix > pistillate flower zone > staminate flower zone). From period I to II, the total amount of all different inflorescence parts decreased, except for the pistillate flower zone that increased (Mann-Whitney, $p > 0.05$). Due to low sampling of the different inflorescence parts, Friedman test showed no significant differences ($p > 0.05$) for total amount of floral scent between the different inflorescence parts.

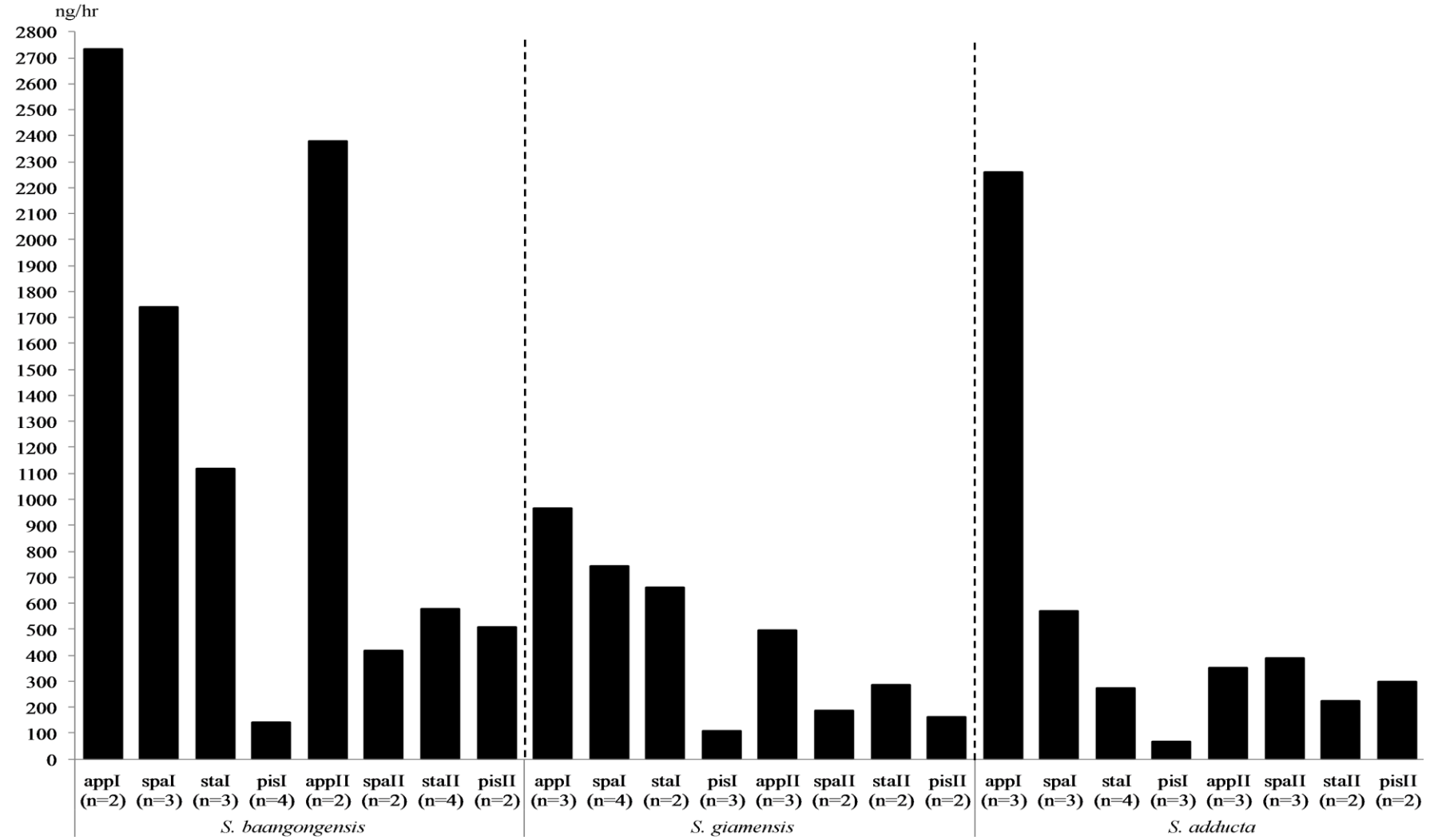

Figure 4.23. Total amount (ng/hr) of the floral scent for appendix (app), pistillate flower zone (pis), spathe (spa) and staminate flower zone (sta) of *Schismatoglottis baangongensis, Schismatoglottis giamensis* and *Schismatoglottis adducta* analyzed by BP20 polar column in split mode. Samples were obtained by dynamic headspace during time period I (0600 – 0800) and II (0815 – 1015) in the course of the pistillate phases of anthesis.

4.4 Discussion

4.4.1 Flowering Mechanisms

In many unisexual-flowered aroids, the persistent spathe is assumed for protecting the infructescenes (Madison, 1979; Mayo *et al.,* 1997; Low *et al.,* 2015). However, the spathe trapping mechanism during pistillate anthesis in this study is probably a temporary shelter for the visiting insects, which protecting the insects to seek food rewards, mating and ovipositing in the spathe chamber. Kumano-Nomura & Yamaoka, (2009) suggested the spathe chamber of one *Homalomena* species is a safe arena for the insect pollinators to mate and seek food rewards. *Cyclocephala* beetles also observed to copulate in the protected floral chamber of *Philodendron solimoesense* A.C. Smith (Gibernau *et al.,* 1999).

Previous study stated that *S. calyptrata* has the spathe blade expands and often bends back abruptly at the onset of staminate anthesis (Bröderbauer *et al.,* 2012). However, this statement is contrary in this study as the spathe limb was abscised at the onset of staminate anthesis. The spathe trapping mechanism for the species of Calyptrata complex was similar with *P. borneense* (Low *et al.,* 2015). However, *P. borneense* only permitted small *Colocasiomyia* flies to access into the pistillate flower zone (Low *et al.,* 2015), excluded larger size *Chaleonus* beetles. Yet the wider space at the spathe constriction flower zone for the species of Calyptrata complex allow larger size insects (*Parastasia* beetles and *Chaleonus* beetles) access to pistillate flower zone.

4.4.2 Pollinator and Visitor

Although more field works were carried out in studied localities in Sarawak, the study of insect pollinators and visitors in this study were based on many examined inflorescence specimens (field pollination investigations and insect collecting) and high natural fruit set were achieved in all investigated species of the Calyptrata complex. Multivariate analyses (Gibernau *et al.,* 2010) suggested that the pollinators of *Schismatoglottis* are distributed between flies and beetles. In this study, *Colocasiomyia* flies are suggested as the main pollinators as they were found with adhered pollen and have the highest visitation number among the insect groups. These behaviours also were observed for *Colocasiomyia* flies pollinator that visited *A. nicolsonii, P. borneense. S. sarikeense* and *Bucephalandra* spp. (Low *et al.,* 2013 & 2015; Wong & Boyce, 2014). While *Colocasiomyia* flies suck on the wet surface of interpistillar staminodes for the species of Calyptrata complex, adhered pollen might be transferred to the wet pistils and set fruits. Field study found high fruit set (88.78 %) in *S. calyptrata*, suggesting that *Colocasiomyia* flies that only remain until the end of the anthesis cross-pollinate the pollen.

Cycreon beetles are considered as the secondary pollinator as it visited all investigated species of Calyptrata complex, except *S. calyptrata* and *S. laxipistillata*. The mean numbers of visiting individuals are abundant (23 – 81) (*S. baangongensis*, *S. giamensis*, *S. caesia* and *S. muluensis*) or very few (1 – 12) and sporadic (*S. roh* Ar2445*, S. pseudoniahensis*, *S. pantiensis, S. adducta* and *S. roh* Ar1240). Up to date, only Low *et al.* (2013 & 2015) reported *Cycreon* beetles visited *P. borneense* and *S. sarikeense* and left with adhered pollen (*S. sarikeense*), yet *Cycreon* beetles were not marked as pollinators since these beetles were not observe to visit the pistillate flower zone of *P. borneense* and *S. sarikeense* (Low *et al.,* 2013

& 2015). However, in the present study, *Cycreon* beetles visit the pistillate zone during pistillate anthesis, left with adhered pollen, carried 6.6 – 18 times more pollen (*S. baangongensis, S. giamensis* and *S. roh* Ar1240) than *Colocasiomyia* flies and their role as the pollinator cannot be ruled out. The *Cycreon* beetles remain poorly investigated and their occurrence in Araceae is very rare. *Cycreon* larvae may probably co-exist with dipteran and nitidulid larvae in the decaying flower parts (A. Kirejtshuk personal communication). So far, only one species – the type *C. sculpturatus* from Palembang, Sumatra was described (Orchymont, 1919). The latest revision by Hansen (1991) did not describe any novel *Cycreon* species as no specimens were accessible and the revision is based on original designation and monotypy of Orchymont (1919). However, this study revealed *Cycreon* beetles not merely confined in Sumatra, but also present in Ambon, Peninsular Malaysia and Sarawak.

Chaloenus beetles and *Parastasia* beetles are considered as opportunistic pollinators. The natural fruit set of *S. baangongensis*, *S. giamensis* and *S. roh* Ar1240 is higher (80.92 – 94.90 %) than fruit set visited by the smallest insects (67.38 – 72.11 %) suggesting *Chaloenus* and *Parastasia* beetles sometimes contribute partially to cross-pollinate the species (at least 12 % of seed set). Pollen adhering also was observed when *Colocasiomyia* flies transferred some adhered pollen onto *Chaloenus* and *Parastasia* beetles. This behaviour was reported here for the first time as pollen transferring among the insect groups has never been reported in Araceae (Beath, 1996; Gibernau *et al.,* 1999 & 2000; Mori & Okada, 2001; García-Robledo *et al.,* 2004; Kumano & Yamaoka 2006; Tung *et al.* 2010; Hoe *et al.* 2011; Takano *et al.,* 2012; Maia *et al.,* 2013; Low *et al.,* 2013 & 2015). In another study in Schismatoglottideae, the visiting behaviours of *Chaloenus* beetles were also similar with the investigated species of the Calyptrata complex. *Chaloenus* beetles were sporadic, destructive, and moved freely

among the spadix zone during pollen release of *A. nicolsonii* and *P. borneense* (Low *et al.*, 2015) and were not suggested as the pollinator. Previous pollination investigations recorded that *P. gestroi and P. nigripennis* carried the resinous pollen of *Homalomena* spp. (Crytocladon supergroup) (Kumano & Yamaoka, 2006; Hoe *et al.,* 2011 & 2016; Tung *et al.,* 2010). As compare to small *Colocasiomyia* flies, *Cycreon* beetles and *Atheta* beetles, the large *P. nigripennis* and *P. gestroi* are rather less effective to carry the powdery pollen of species of Calyptrata complex. Resinous pollen may ensure better sticked pollen on larger size scarab beetle was reported (Gibernau & Barabé, 2002). Maia *et al.* (2013) and Sannier *et al*. (2009) reported that poor pollenkitt (powdery pollen) of Araceae is inadequate to be carried out by the glabrous body surface of large scarab beetle. Kumano and Yamaoka (2006) suggested that the sticky pollen of investigated *Homalomena* could not properly adhere to the body of *Colocasiomyia* flies.

Atheta staphylinid beetles are the visitors in all species of Calyptrata complex in Sarawak, except *S. muluensis.* The mean number of visiting individuals was very low (1 – 9), sporadic (*S. baangongensis*, *S. roh* Ar2445*, S. giamensis, S. pseudoniahensis, S. adducta* and *S. roh* Ar1240) and with no adhered pollen. These beetles are presuming to target eggs and larvae of *Colocasiomyia* flies or other insects that breed on the inflorescence as they were mostly moving among the pistils (Takano *et al.,* 2012). Field pollination studies in Araceae mostly recorded staphylinid beetles as the insect visitors (Gibernau *et al.,* 1999 & 2000; García-Robledo *et al.,* 2005 & 2004; Maia *et al.,* 2010; Tung *et al.,* 2010; Chartier *et al.,* 2011; Hoe *et al.,* 2011; Low *et al.,* 2013 & 2015) or predators (García-Robledo *et al.,* 2005; Takano *et al.,* 2012). However, in *S. muluensis, Atheta* beetles are secondary pollinators as these beetles were not sporadic and left with adhered pollen. Further investigation (pollen count test) is

required to strengthen the pollinator status of *Atheta* in *S. muluensis*.

Trigona bees, Pteromalid wasps and Chironomid midges are considered as visitors. During pistillate anthesis, *Trigona* bees only visited the spathe limb of *S. muluensis* for a short while and were never observed in the pistillate flower zone. Previous studies reported *Trigona* bees collected pollen or resin from the inflorescences but did not visit the pistillate flower zone (Gibernau *et al.*, 1999; Hoe *et al.,* 2016). Pteromalid wasps and Chironomid midges were mostly seeking for breeding site on staminate flower zone of *S. calyptrata* and left during inter-anthesis anthesis. Pteromalid wasps are parasitic small hymenoptera that presumely targeting larvae and eggs of other visited insects (M.T. Kimura, personal communication) or targeting the seed of the infructescences (Gibernau *et al.,* 2002). So far, chironomid midges have never been reported to visit the inflorescence of the tropical aroids (M. Gibernau personal communication).

In this study, no specific pollinators or specific opportunistic pollinators were suggested due to *Cycreon* beetles, *Colocasiomyia* flies and *Chaloenus* beetles carried other unidentified pollen (**Figure 4.15**). *Parastasia* beetles (*P. bimaculata* and *P. gestroi*) are known to pollinate *Homalomena* spp. (Hoe *et al.,* 2016).

In Araceae, beetle pollinator is general associated with the presence of edible staminodes or pistillodes since the voracious phytophagous insects can damage the inflorescence structures (Young, 1986; Gottsberger, 1999; Kumano & Yamaoka, 2006; Tung *et al.,* 2010; Hoe *et al.,* 2011 & 2016). In this study, interpistillar staminodes present in the investigated species of the Calyptrata complex, but it appears that the main pollinators are *Colocasiomyia* flies.

Colocasiomyia flies pollinated inflorescence of *Alocasia* (Yafuso, 1993; Miyake & Yafuso, 2003 & 2005) and *Colocasia* (Carson & Okada, 1980; Ivancic *et al.,* 2008) that does not have interpistillar staminodes. It is hypothesis that 1) the investigated species of the Calyptrata complex have a mixed fly-beetle pollinated system (Chouteau *et al.,* 2008; Gibemau *et al.,* 2010); 2) ancestral beetle-pollinated character present in a newly fly-pollinated system and 3) new floral characters in an ancestral fly-pollinated plant group, with the possibility to reduce towards beetle pollination.

4.4.3 Breeding

The breeding behaviour of *Colocasiomyia* fly has been reported in aroid inflorescences (Carson & Okada, 1980; Okada & Yafuso, 1989; Yafuso & Okada, 1990; Yafuso, 1993; Yafuso, 1994; Miyake & Yafuso, 2003 & 2005; Takenaka *et al.,* 2006; Takano *et al.,* 2012; Fartyal *et al*., 2013; Low *et al.,* 2015). The preliminary breeding test in this study showed that *Colocasiomyia* flies oviposited eggs on the spadix and adult *Colocasiomyia* flies emerged from the spathe (*S. roh* Ar2445) and the pistillate flower zone (*S. roh* Ar2445 and *S. giamensis*). Previous studies reported that adult *Colocasiomyia* flies emerged from the appendix, the staminate and the pistillate flower zones of the inflorescence of *A. odora* C.Koch. (Yafuso, 1994) and the staminate and the pistillate flower zone of *A. cucullata* Schott (Miyake & Yafuso, 2005). This study showed that *Colocasiomyia* not only seek for food rewards and mating partners, but also seek for ovipositing site in different parts of the inflorescence in the species of the Calyptrata complex. No synhospitalic and cohabitating (Takano *et al.,* 2011) breeding habit are identified. Further studies in identifying (eggs, larvae and emerged flies), counting (eggs and larvae) and measuring the survival rate of the emerged *Colocasiomyia* flies are required.

4.4.4 Thermogenesis

The thermogenesis for the appendix and staminate flower zone of *S. adducta, S. calyptrata, S. giamensis, S. pseudoniahensis* and *S. roh* Ar1240 were biphasic, which was similar to a *Homalomena* species in perhumid lowland tropical forest (Kumano & Yamaoka, 2006). Like other tropical Araceae (*Philodendron* and *Homalomena*) the temperature patterns is biphasic (Gibernau *et al.,* 2000; Barabé *et al.,* 2002b; Gibernau & Barabé, 2002; Kumano & Yamaoka, 2006; Maia *et al.,* 2010; Pereira *et al.,* 2014) and the two thermogenic zones (appendix and staminate flower zone) are synchronous (Barabé *et al.,* 2002b). These floral zones probably show the same roles in relation to thermogenesis. This is contrary to several temperate Araceae (*Arum, Dracunculus* and *Sauromatum*) where the thermogenic patterns of the appendix and staminate flower zones are not synchronous during the anthesis (Meeuse and Raskin, 1988; Bermadinger-Stabentheiner & Stabentheiner, 1995; Seymour & Schultze-Motel, 1999).

The first temperature peak during pistillate anthesis correspond with the spathe opening movement (Albre *et al.,* 2003) and promoting the scent emission to greater distance for attracting the pollinator (Meeuse & Raskin, 1988; Gottsberger & Silberbauer-Gottsberger, 1991; Mayo *et al.,* 1997; Gibernau & Barabé, 2002; Gibernau *et al.,* 2004; Kumano & Yamaoka, 2006). The larger heat production on appendix and staminate flower zone is presumably to support high energetic activities (courtship, mating and fighting), whereas the lesser heat production on pistillate flower zone supports low energetic activities (eating and sucking on the surface of the interpistillar staminodes, feeding on the liquid secreted from the stigma). Previous studies reported the warm inflorescence could reduce the energy cost of mating and eating activities of the *Cyclocephala colasi* scarab beetles (Seymour *et al.,* 2003 &

2009). Dieringer *et al*. (1998) suggested *C. caelestis* regulated the heat at night during cool temperature was important for mating and feeding activities. Warming could have a negative effect on stigma physiology and ovule viability by the visited insects. The pistils are protected in general within the spathe chamber and thus with a slightly higher temperature than ambient air. The lowest temperature increase in pistillate flower zone is presumed with the floral chamber space could be already partly warm by the upper heating parts of the spadix.

At the onset of staminate anthesis, the second temperature peak is presumed to support the warming (Gibernau & Barabé, 2002; Albre *et al.,* 2003; Seymour *et al.,* 2003), pollen release (Seymour & Schultze-Motel, 1997 & 1999; Gibernau *et al.,* 2000; Gibernau & Barabé, 2002; Albre *et al.,* 2003) and departing activities of the insects (Gibernau *et al.,* 2000; Albre *et al.,* 2003). The large *Parastasia* beetles and *Chaloenus* beetles competed for the space on the appendix is probably in order to gain more heat reward before departing. One study (Seymour *et al.,* 2009) showed that when *Cyclocephala colasi* scarab beetle remained on the spadix of *P. solimoesense* that heated up to 29 ºC, these beetles were found with 28 – 30 ºC of thoracic surface temperatures before departing, and the ambient temperature was 23 ºC.

4.4.5 Relationship between Interpistillar Staminodes and Insect Visitors

It is presumed that species of Calyptrata complex with less number of interpistillar staminodes is fly-pollinated, whereas the species with higher number of interpistillar staminodes attracting the *Parastasia* beetles and *Chaloenus* beetles. *Schismatoglottis calyptrata* was found pollinated by *Colocasiomyia* flies. Unlike beetles, *Colocasiomyia* flies do not chew the interpistillar staminodes and probably the inflorescences of *S. calyptrata* bear the fewest number of interpistillar staminodes (6 – 12 per inflorescence). Pollination studies also showed

several tropical aroids (*Alocasia* and *Colocasia*) that do not have interpistillar staminodes were pollinated by the *Colocasiomyia* flies (Carson & Okada, 1980; Yafuso, 1993; Miyake & Yafuso, 2003 & 2005; Ivancic *et al.,* 2008). *Homalomena* spp. that lacking interpistillar staminodes (Punctulata Supergroup and Homalomena Supergroup) are only attended by the *Colocasiomyia* flies (P.C.Boyce, pers. obs.).

The species of the Calyprata complex in Sarawak that bear moderate to many number of interpistillar staminodes (100 – 469) (*Schismatoglottis roh* Ar1240, *S. roh* Ar2445, *Schismatoglottis baangongensis* and *S. muluensis*) were visited by the *Chaleonus* beetles and *Parastasia* beetles. It is probably these beetles also contribute in transferring the pollen, in return the inflorescences bear many interpistillar staminodes. The interpistillar staminodes are rich in protein and carbohydrate and are the food reward structure for scarab beetles (Young, 1986). Scarab pollinators (*Cyclocephala* and *Parastasia*) or *Chaleonus* insect visitors (Young, 1986; Kumano & Yamaoka, 2006; Tung *et al.,* 2010; Hoe *et al.,* 2011 & 2016) visited several aroids (*Dieffenbachia* and *Homalomena*) that have many numbers of interpistillar staminodes.

The Calyptrata complex species in Peninsular Malaysia visited by one destructive beetle – *Chaloenus* spp. and bear lower number of interpistillar staminodes than the Calyptrata complex species in Sarawak. It is suggested that the lesser number of interpistillar staminodes is sufficient as the food reward for *Chaleonus* spp.. The reduce number of interpistillar staminodes may probably reduce the energy investment of the inflorescences.

4.4.6 Scent-Pollinator

The inflorescence sample analyzed by BPX-5 intermediate polar column (four compounds) detected lesser compounds than BP20 polar column (nine compounds). All identified VOCs from BPX-5 intermediate polar column were detected in BP20 polar column. Only identified VOCs from BP20 polar column are discussed here.

The inflorescences of all investigated species of Calyptrata Complex emitted all four identified ester VOCs. This correlated with the reminiscent of intensified esteric scent described in field pollination investigations. To date, 3-butenoic acid, 3-methyl-, methyl ester only reported from the petals of *Robinia pseudoacacia* L. (Fabaceae) (Aronne *et al.,* 2014). 3-buten-1-ol, 3-methyl- (< 2.7 %) was present in floral of *Narcissus* spp. (Dobson *et al*., 1997) and sweet pea fruit of *Lathyrus odoratus* L. (Porter *et al*., 1999). Methyl benzoate is commonly found in various angiosperms families as minor relative amount (< 5 %) in Araceae (Schwerdtfeger *et al.*, 2002), Asteraceae, Caryophyllaceae, Polemoniaceae, Rubiaceae, Verbenaceae (Andersson *et al*., 2002) or as major relative amount (44.53 – 91.6 %) in Amaryllidaceae (Dobson *et al*., 1997), Caryophyllaceae (Jürgens, *et al*., 2002) and Nyctaginaceae (Levin *et al*., 2001). 2-Butenoic acid, 3-methyl-, methyl ester was found in several angiosperms families: Cycadaceae (Pellmyr *et al.,* 1991), Asteraceae (Pham-Delegue *et al.,* 1989), Amaryllidaceae (Dobson *et al.,* 1997) and Arecaceae (Knudsen, 1999). The roles of pollination attraction in these ester compounds were not further discussed except Dobson *et al.* (1997) revealed *Narcissus assoanus* Dufour and *N. jonquilla* L. that emitted 2-Butenoic acid, 3-methyl-, methyl ester and other isopentenoid ester and benzenoid ester VOCs attracted the similar Sphingidae (Lepidoptera) moths. It is probably that the species of Calyptrata complex emitted ester group VOCs to attract the *Colocasiomyia* flies pollinator.

The different inflorescence parts of all three investigated species of Calyptrata complex (*S. adducta, S. baangongensis* and *S. giamensis*) emitted similar identified ester VOCs from its inflorescence respectively except two additional compounds: unidentified ester compound 1 and indole (**Table 4.7**). These two compounds were assumed to be VOCs induced by the spathe cutting damage (Kumano & Yamaoka, 2006).

The appendix in investigated species of Calyptrata complex releases the highest amount and the highest number of VOCs (four VOCs). It is possible that all different inflorescence parts contribute in pollinator attraction, especially the appendix. Miyake and Yafuso (2003) tested different inflorescence parts and found the appendix of *Alocasia odora* is the main olfactory attractant. The appendix attracted the highest number of *Colocasiomyia* pollinators, followed by the staminate flower zone + upper sterile flower zone, and the pistillate flower zone + lower sterile flower zone in the field and the laboratory (Miyake & Yafuso, 2003). For appendix lacking *Homalomena* sp., Kumano and Yamaoka (2006) recorded that more beetles (*Parastasia* and chrysomelids) were attracted to the entire spadix inflorescence than the spathe.

From period I to period II, only the pistillate flower zone increased its total amount of floral scent released, whereas floral scent from other inflorescence parts were reduced. At this period, most insect (*Colocasiomyia* flies, *Cycreon* beetles, staphylinid beetles) remain in the lower spathe chamber. It is presume that more adhered pollen can be transferred onto the pistils by maintained the insects in the lower spathe chamber.

The scent patterns have weak interaction effect (two-way PERMANOVA, $p < 0.047$) with factor 'opportunistic pollinator: present of *Parastasia* beetle'. This weak interaction effect probably explain only four Calyptrata species (*S. baangongensis, S. roh* Ar2445*, S. muluensis* and *S. roh* Ar1240) in Sarawak visited by *Parastasia* beetles, excluding other investigated species of Calyptrata complex.

CHAPTER 5

MOLECULAR ANALYSES AND CHARACTERS MAPPING

5.1 Introduction

Molecular studies based on combined nuclear (ITS) or plastid regions (*trn*L – *trn*L-F, *mat*K, *trn*H – *psb*A) in tribe Schismatoglottideae were carried out to establish the inter-tribal and inter-generic relationships in Schismatoglottideae (Wong *et al.,* 2010; Low *et al.,* 2011; Wong, 2013 and Low *et al.,* 2014). Within *Schismatoglottis,* so far, only the Nervosa complex and one species of *Schismatoglottis* Calyptrata complex were investigated (Barabé, *et al.,* 2004; Wong *et al*., 2010). In this chapter, the phylogeny of 22 species of *Schismatoglottis* Calyptrata complex were investigated using ITS and *mat*K regions. The phylogenetic tree based on *mat*K was then reduced to include ten taxa (eleven accessions) of Calyptrata complex was mapped with locality (1), insect visitors (4), floral scent (2) and morphological characters (4).

5.2 Materials and Methodology

5.2.1 Taxa Sampling

A total of 21 taxa (22 accessions) of *Schismatoglottis* Calyptrata complex, two taxa of *Schismatoglottis* Asperata group and one taxon of *Phymatarum borneense* were included as ingroup taxa. Sequences of 21 taxa (22 accessions) of *Schismatoglottis* Calyptrata complex were newly generated and submitted to GenBank with the accession numbers from KP748473 to KP748516. *Philonotion americanum* (A.M.E. Jonker & Jonker) S.Y.Wong & P.C.Boyce and *Cryptocoryne longicauda* Becc. ex. Engl. were chosen as outgroups for the study. The

outgroups were selected based on Low *et al.* (2013) and Wong *et al.* (2013). Collection locality, GPS, voucher information and GenBank accession numbers are provided in **Table 5.1.**

5.2.2 DNA Extraction and Polymerase Chain Reaction (PCR) Amplification and Sequencing

Total genomic DNA was extracted from the fresh young leaf tissues using a modified 2 x CTAB protocol (Doyle & Doyle, 1987) with the addition of PVP (Polyvinylpyrrolidone) (Wong *et al*., 2013). The nuclear ITS region and plastid *mat*K gene region were targeted for polymerase chain reaction (PCR) amplification. Primers used were: ITS 1F (5'-GAGGAAGGAGAAGTCGTAACAAGG-3') (White *et al.,* 1990), ITS 1R (5'-ACTTGCGTTCAAAGATTCGATGG-3') (White *et al.,* 1990), ITS 3F (5'-GCATCGATGAAGAACGTAGC-3') (White *et al.,* 1990), ITS 4R (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.,* 1990); *mat*K 19F (5'-CGTTCTGACCATATTGCACTATG-3') (Gravendeel *et al.,* 2001), *mat*K 2R (5'-AACTAGTCGGATGGAGTAG-3') (Steele & Vilgalys, 1994), *mat*K 390F (5'-CGATCTATTCATTCAATATTTC-3') (Cuénoud *et al.,* 2002) and *mat*K 1326R (5'-TCTAGCACACGAAAGTCGAAGT-3') (Cuénoud *et al.,* 2002). PCR Amplifications for ITS *mat*K regions were performed in a Bio-Rad thermal cycler with the PCR profile set to 2 min at 95 ºC, followed by 40 cycles of 95 ºC for 1 min, 50 – 54 ºC for 1 min, and 72 ºC for 2 min, with a final extension of 72 ºC for 10 min. The PCR products were viewed on a 1.5 % agarose gels, and purified using a PCR purification kit (Fermentas, Vilnius, Lithuania). Purified products were viewed again using a 1 % agarose gel, and if a single clear band was present the products were sent for sequencing to Beijing Genomics Institute Laboratories, Guangdong,

Table 5.1. List of specimens investigated: Taxon, accession number, GPS, collection locality, gene region, GenBank accession number and collector.

Taxon	Accession number	GPS	Collection locality	Gene region	GenBank accession number	Collector
Cryptocoryne longicauda Becc. ex. Engl.	Ar1366 (SAR)	04.02 N; 114.48 E	Malaysia, Sarawak, Miri, Marudi, Long Lama, Mulu National Park, Deer Cave trail	ITS *mat*K	KM433709 KM433722	L.S.Tung
Philonotion americanum (A.M.E.Jonker & Jonker) S.Y.Wong & P.C.Boyce	BOGNER 2911	-	French. Guiana	ITS *mat*K	JN544445 GQ220908	J. Bogner
Phymatarum borneense Ar1931	Ar1931 (SAR)	04. 02 N; 114. 48 E	Malaysia, Sarawak, Miri Mulu National Park, Trail to Deer Cave	ITS *mat*K	JN544436 GQ220900	P. C. Boyce *et al.*
Schismatoglottis calyptrata cf.	Ar382 (SAR)	01.44 N; 113.28 E	Malaysia, Sarawak, Kapit, Namga Gaat, Rejang Wood Concession	ITS *mat*K	KP748496 KP748474	P.C.Boyce *et al.*
Schismatoglottis calyptrata cf.	Ar1638 (SAR)	00.57 N; 110.30 E	Malaysia, Samarahan, Serian, Mongkos, Kampung Batuh, Darud Silabur	ITS *mat*K	KP748500 KP748478	S.K.Paru
Schismatoglottis calyptrata cf.	Ar3586 (SAR)	03.18 N; 101.41 E	Malaysia, Selangor, Sungai Tua, Taman Negeri Selangor	ITS *mat*K	KP748505 KP748483	P.C.Boyce *et al.*
Schismatoglottis calyptrata cf.	Ar3615 (SAR)	01.14 N; 110.17 E	Malaysia, Sarawak, Kuching, Padawan, Annah Rais	ITS *mat*K	KP748506 KP748484	P.C.Boyce & S.Y.Wong
Schismatoglottis calyptrata cf.	Ar3662 (SAR)	02.16 N; 111.50 E	Malaysia, Sarawak, Sibu, Siburan, Sentosa, Jalan Ek	ITS *mat*K	KP748507 KP748485	A.P.J.Ting

			Dee			
Schismatoglottis calyptrata cf.	Ar3673 (SAR)	00.59 N; 110.33 E	Malaysia, Sarawak, Samarahan, Serian, Kampung Mayang	ITS *mat*K	KP748508 KP748486	P.C.Boyce & S.Y.Wong
Schismatoglottis calyptrata cf.	Ar3679 (SAR)	01.37 N; 111.35 E	Malaysia, Sarawak, Betong, Spaoh, Jalan Ulu Paku, Nanga Penum	ITS *mat*K	KP748509 KP748487	P.C.Boyce & S.Y.Wong
Schismatoglottis calyptrata cf.	Ar3956 (SAR)	00.58 S; 11.30 E	Indonesia, Kalimantan, Kalimantan Barat, Melawi	ITS *mat*K	KP748510 KP748488	E, K.Nakamoto
Schismatoglottis calyptrata cf.	Ar4023 (SAR)	01.11 N; 111.40 E	Malaysia, Sarawak, Sri Aman, Lubok Antu, Engkilili, Tempat Rekreasi Batu Ngabau	ITS *mat*K	KP748499 KP748477	Y.C.Hoe
Schismatoglottis calyptrata cf.	Ar4096 (SAR)	01.57 N; 111.30 E	Malaysia, Sarawak, Sarikei, Sungai Lepong	ITS *mat*K	KP748511 KP748489	P.C.Boyce & S.Y.Wong
Schismatoglottis calyptrata (Roxb.) Zoll. & Moritzi	Ar4270 (SAR)	03.36 S; 128.19 E	Indonesia, Maluku, Ambon, Salahutu, East Suli	ITS *mat*K	KP748512 KP748490	Y.C.Hoe
Schismatoglottis calyptrata cf.	Ar4651 (SAR)	01.46 N; 109.43 E	Malaysia, Sarawak, Kuching, Sematan, Kampung Temaga Dayak, Sungai Temaga, trail to Gunung Pueh	ITS *mat*K	KP748515 KP748493	P.C.Boyce & S.Y.Wong
Schismatoglottis muluensis M.Hotta	Ar1941 (SAR)	04.02 N; 114.49 E	Malaysia, Sarawak, Miri, Marudi, Long Lama, Mulu National Park, Deer Cave trail	ITS *mat*K	KP748502 KP748480	P.C.Boyce *et al.*
Schismatoglottis pseudoniahensis S.Y.Wong & Y.C.Hoe	Ar4666	03.49 N; 113.45 E	Malaysia, Sarawak, Miri, Niah Suai, Niah National Park	ITS *mat*K	KP748516 KP748494	Y.C.Hoe (SAR)
Schismatoglottis	Ar2588	01.20 N;	Malaysia, Sarawak, Kuching,	ITS	KP748504	P.C.Boyce &

baangongensis S.Y.Wong & Y.C.Hoe	(SAR)	110.20 E	Padawan, Sikog Village	*mat*K	KP748490	S.Y.Wong
Schismatoglottis roh Ar2445 S.Y.Wong & Y.C.Hoe	Ar2445 (SAR)	01.22 N; 110.07 E	Malaysia, Sarawak, Kuching, Bau, Fairy cave	ITS *mat*K	KP748495 KP748473	P.C.Boyce & S.Y.Wong
Schismatoglottis giamensis S.Y.Wong & Y.C.Hoe	Ar2549 (SAR)	01.19 N; 110.16 E	Malaysia, Sarawak, Kuching, Siburan, Kampung Giam	ITS *mat*K	KP748503 KP748481	P.C.Boyce & S.Y.Wong
Schismatoglottis caesia S.Y.Wong & Y.C.Hoe	Ar4332 (SAR)	04.52 N; 102.26 E	Malaysia, Kelantan, Gua Musang, Kuala Koh, Taman Negara Kuala Koh	ITS *mat*K	KP748514 KP748492	Y.C.Hoe
Schismatoglottis pantiensis S.Y.Wong & Y.C.Hoe	Ar4322 (SAR)	01.48 N; 103.51 E	Malaysia,. Johor, Kota Tinggi, Hiking trail to Hutan Simpan Panti	ITS *mat*K	KP748513 KP748491	Y.C.Hoe
Schismatoglottis adducta S.Y.Wong & Y.C.Hoe	Ar1632 (SAR)	01.06 N; 111.30 E	Malaysia, Sarawak, Sri Aman, Taman Rekreasi Sungai Raya,	ITS *mat*K	KP748498 KP748476	P.C.Boyce *et al.*
Schismatoglottis laxipistillata S.Y.Wong & Y.C.Hoe	Ar4331 (SAR)	05.44 N; 100.26 E	Malaysia, Kedah, Merbok, Hutan Lipur Rekreasi Tupah	ITS *mat*K	KP748501 KP748479	Y.C.Hoe
Schismatoglottis roh Ar1240 S.Y.Wong & Y.C.Hoe	Ar1240 (SAR)	01.24 N; 110.08 E	Malaysia, Sarawak, Kuching, Bau, Wind cave	ITS *mat*K	KP748497, KP748475.	P.C.Boyce & J.Kisai
Schismatoglottis nervosa	Ar944 (SAR)	01.23 N; 110.07 E	Malaysia, Sarawak, Kuching, Bau, Gunung Bidi	ITS *mat*K	JX076800 JN570742	P.C.Boyce & Jeland ak Kisai
Schismatoglottis ulusarikeiensis	Ar1579 (SAR)	01 55 05.4N 111 29 35.8E	Malaysia, Sarawak, Sarikei, Ulu Sarikei	ITS *mat*K	JX857115 JN570746	P.C.Boyce *et al.*

China. Sequencing reactions were carried out using the same primer pairs during PCR amplification and the ABI PRISM BigDye® Terminator Version 3.0 Cycle Sequencing Kit on an ABI® 377 DNA automated sequencer (Applied Biosystem, Foster City, CA, USA).

5.2.3 Sequence Alignment and Phylogenetic Analyses

Newly generated sequences were assembled using BioEdit version 5.0.6 (Hall, 1999) and manually adjusted (**Appendix 6**). The most suitable nucleotide substitution model for gene regions *mat*K, ITS and ITS + *mat*K was selected in jModeltest ver. 0.1.1 (Posada, 2008) using Akaike information criterion (AIC; Akaike, 1974). The jModeltest identified that the Transversion Model plus Gamma (TVM+G) as best fit for gene *mat*K, Kimura 3-parameter (TPM1uf+G) as best fit for gene ITS and K81 (Kimura 1981) (TPM1+I+G) as best fit for gene ITS + *mat*K.

Maximum likelihood (ML) analyses were carried out in PhyML 3.0 (Guindon *et al.,* 2010) using the HKY85 model (Hasegawa-Kishino-Yano 85) with estimated gamma shape parameters and optimized topology and branch lengths (Bilgili *et al.,* 2016). ML bootstrap values were obtained by running 10,000 replicates. Bayesian phylogenetic analyses were performed with MrBayes ver.3.1.2 (Huelsenbeck & Ronquist, 2001). Markov chain Monte Carlo (MCMC) was repeated twice to assure parameter convergence. The MCMC algorithm was run for 2,000,000 (*mat*K), 2,000,000 (ITS) and 2,000,000 (ITS + *mat*K) generations with one cold and three heated chains, starting from random trees and sampling one out of every 100 generations (Low *et al.,* 2013; Wong *et al.,* 2013). Convergence was assessed by using the standard deviation of split frequencies as convergence index with values 0.005 – 0.01. The first 10 % of trees were discarded as burn-in. Remaining trees were used to construct 50 %

majority rule consensus trees. In interpreting phylogenetic confidence, ML bootstrap support: 50 – 74 % represents weak support, 75 – 84 % moderate support, and 85 – 100 % represents strong support. Bayesian posterior probability (BPP): 0.50 – 0.74 represents weak support, 0.75 – 0.94 represents moderate support, and 0.95 – 1.00 represents strong support. ML < 50% and BPP < 0.50 are not showed on the tree (Low *et al.,* 2013; Wong *et al.,* 2013).

5.2.4 Characters Mapping

Based on the availability of the data for the species of the Calyptrata complex, the ten taxa (eleven accessions) phylogenetic tree (*mat*K) was mapped with 11 characters (locality, insect visitors, floral scent and morphology) (**Figure 5.3**). The character states were reconstructed by based on the methods in Wong *et al.* (2013) for: (1) Locality: 0 = Sarawak; 1 = Peninsular Malaysia; 2 = Ambon; (2) *Colocasiomyia* spp.: 0 = present; 1 = absent; (3) Chironomid midge: 0 = present; 1 = absent; (4) *Cycreon* sp.: 0 = present; 1 = few (1 – 12); 2 = abundance (23 – 81); (5) *Parastasia* spp.: 0 = present; 1 = absent; (6) 3-butenoic acid, 3-methyl-, methyl ester and 2-butenoic acid, 3-methyl-, methyl ester: 0 = present; 1 = absent; 2 = not sampled; (7) 3-buten-1-ol, 3-methyl: 0 = present; 1 = absent; 2 = not sampled; (8) Pistils: 0 = crowded; 1 = crowded at proximal but laxly at distal; 2 = laxly; (9) Number of interpistillar staminodes: 0 = very few (6 – 12); 1 = few (13 – 66); 2 = average to many (80 – 469); (10) Height of interpistillar staminodes in comparison with associated pistils: 0 = equaling; 1 = slightly exceeding (0.2 – 0.5x); 2 = up to double the height (1 – 2x); (11) Interstice: 0 = slender than pistillate and staminate flower zone, partially naked, comprised ca two whorls of flattened unequal spheroid staminodes at distal, intergrading into the lower staminate flower zone, pistillodes flattened at proximal, few intergrading into the lower staminate flower zone; 1 = Slender than pistillate and staminate zone, partially naked, comprised 2 – 3 whorls of flattened

disc – like staminodes, intergrading into the lower staminate zone, pistillodes flattened at proximal; 2 = Slender than pistillate and staminate zone, partially naked, flattened trapezoid staminodes at proximal and distal, pistillodes flattened at proximal; 3 = Weakly slender than pistillate and staminate zone, partially naked, comprised 2 – 5 whorls of clavate staminodes that resemble interpistillar staminodes, slightly laxly packed, staminodes and pistillodes not impressed; 4 = Slender than pistillate and staminate zone, partially naked, comprised ca 1 whorls of flattened unequal spherical staminodes, intergrading into the lower staminate zone, pistillodes flattened at proximal; 5 = Slender than pistillate and staminate zone, partially naked, few flattened irregular spheroid staminodes closely packed at proximal, partially intergrading into the lower staminate zone, few pistillodes flattened at proximal; 6 = Slender than pistillate and staminate zone, partially naked, comprised ca 2 whorls of flattened clavate staminodes that resemble interpistillar staminodes, intergrading into the lower staminate zone, pistillodes flattened at proximal; 7 = Slender than pistillate and staminate zone, partially naked, comprised 2 – 5 whorls of flattened spheroid staminodes at distal, intergrading into the lower staminate zone, pistillodes flattened at proximal, few pistillodes intergrading into the lower staminate zone; 8 = Slender than pistillate and staminate zone, not naked, comprised 2 – 4 whorls of clavate staminodes, densely packed, staminodes and pistillodes not impressed; 9 = Weakly slender than pistillate and staminate zone, not naked, comprised 10 – 12 whorls of sub-globose staminodes that resemble staminodes at appendix, densely packed, staminodes and pistillodes not impressed. Character matrices were analysed in Mesquite ver. 2.7.4 (Maddison and Maddison, 2010) using parsimony (unordered; Fitch 1971). The matrices were traced onto the maximum likelihood tree from PhyML analysis based on the *mat*K region.

5.3 Results and Discussion

5.3.1 Matrix Characteristics

The total aligned nucleotides for the ITS region has 802 bp, 156 (19.45 %) variable characters, 57 (7.17 %) informative characters, a tree length of 292 steps, 0.887 consistency index (CI), 0.723 retention index (RI) and 2449.38 (BPP)/ 2431.01 (ML) log likelihood value; the total aligned nucleotides for the *mat*K region has 1618 bp, 101 (6.24 %) variable characters, 24 (148 %) informative characters, a tree length of 140 steps, 0.214 consistency index (CI), 0.8533 retention index (RI) and 3137.55 (BPP)/ 3134.55 (ML) log likelihood value; the total aligned nucleotides for the ITS-*mat*K region has 2441 bp, 253 (10.36 %) variable characters, 87 (3.55 %) informative characters, a tree length of 448 steps, 0.8705 consistency index (CI), 0.7339 retention index (RI) and 6013.60 (BPP)/ 6019.48 (ML) log likelihood value. The incongruence length difference (ILD; Farris *et al.,* 1994) test between the ITS and *mat*K regions were not congruent (P = 0.01). Therefore, the two regions were not combined for further analyses.

5.3.2 Phylogenetic Analyses

The tree generated from *mat*K (**Figure 5.1**) was selected as it produced a well resolved tree topology. *Phymatarum borneense* formed polytomy with Asperata group and Calyptrata complex and these taxa were unresolved. *Schismatoglottis* spp. were separated into two clades: Asperata group and Calyptrata complex. The species of the Calyptrata complex were separated into two clades (1.00/75), clade A and clade B. Within clade A, species of Calyptrata complex from Peninsular Malaysia (*S. laxipistillata* (Ar4331), *S. pantiensis* (Ar4322), *S. caesia* (Ar4332) and *S. calyptrata* cf. (Ar3586)) formed a strong supported clade

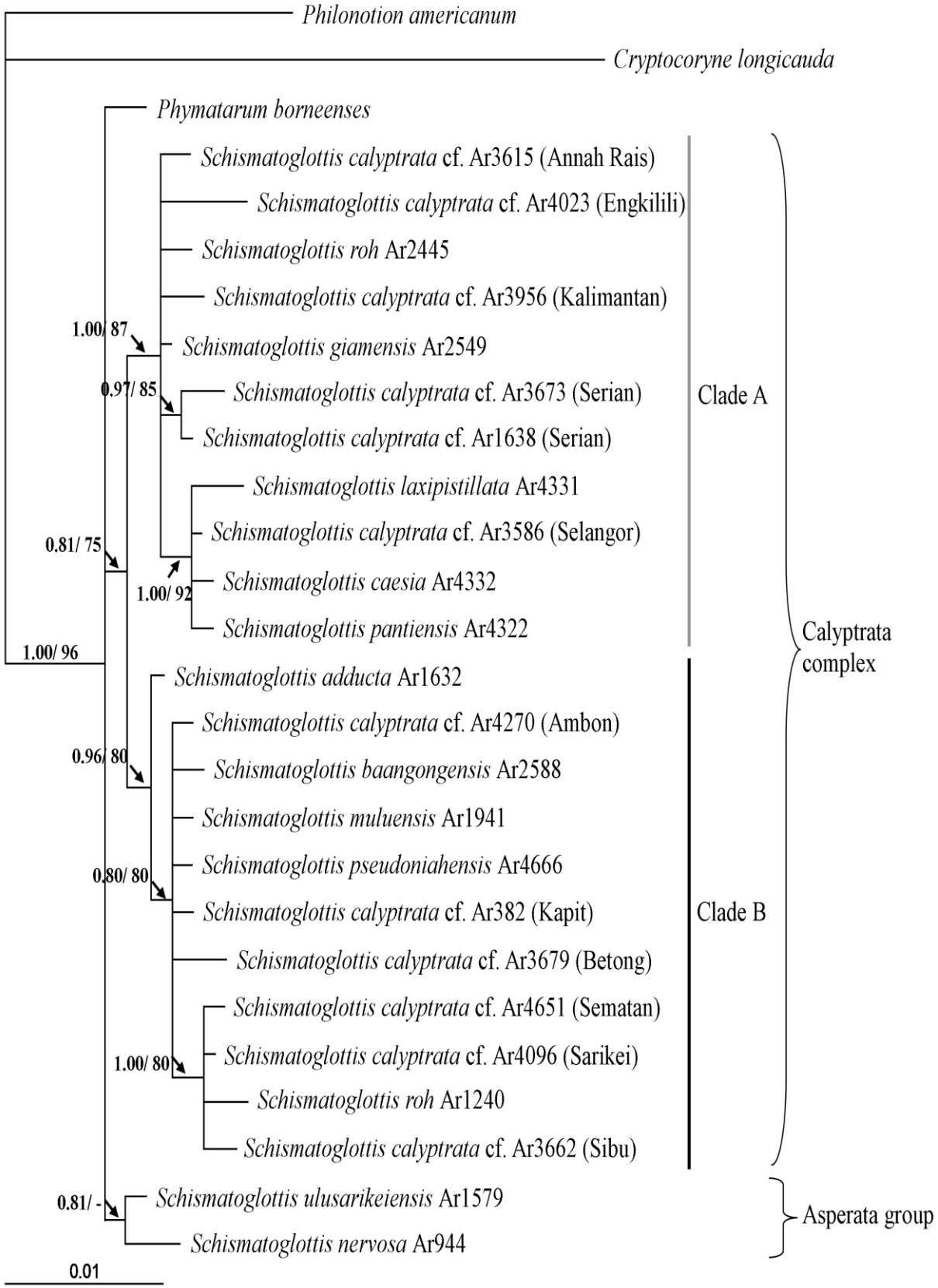

Figure 5.1. Bayesian 50 % majority rule consensus tree obtained using the *mat*K region. Bayesian posterior probabilities > 0.50 and bootstrap values > 50 % (maximum likelihood) are shown below the branches.

(1.00/92). *Schismatoglottis calyptrata* cf. (Ar3673) formed a strong clade with *S. calyptrata* cf. (Ar1638) (0.97/85). Clade B was moderately supported (0.97/80). *Schismatoglottis calyptrata* cf. (Ar4651) formed a strong clade with *S. calyptrata* cf. (Ar4096), *S. roh* (Ar1240), and *S. calyptrata* (Ar3662) (1.00/80).

The trees generated from ITS (**Figure 5.2**) produced a less resolved tree topology. *Phymatarum borneense,* Asperata group and Calyptrata complex were placed within a clade (0.99/88). *Phymatarum borneense* and Asperata group formed a moderately supported clade (0.77/99). All the other taxa included formed polytomies which resulted in the relationships among these taxa were unresolved. However, several groups were supported: *S. calyptrata* (Ar1638), *S. calyptrata* (Ar3673) and *S. calyptrata* (Ar4651); *S. laxipistillata* (Ar4331) and *S. calyptrata* (Ar3586); *S. roh* (Ar2445) and *S. calyptrata* (Ar3662) respectively.

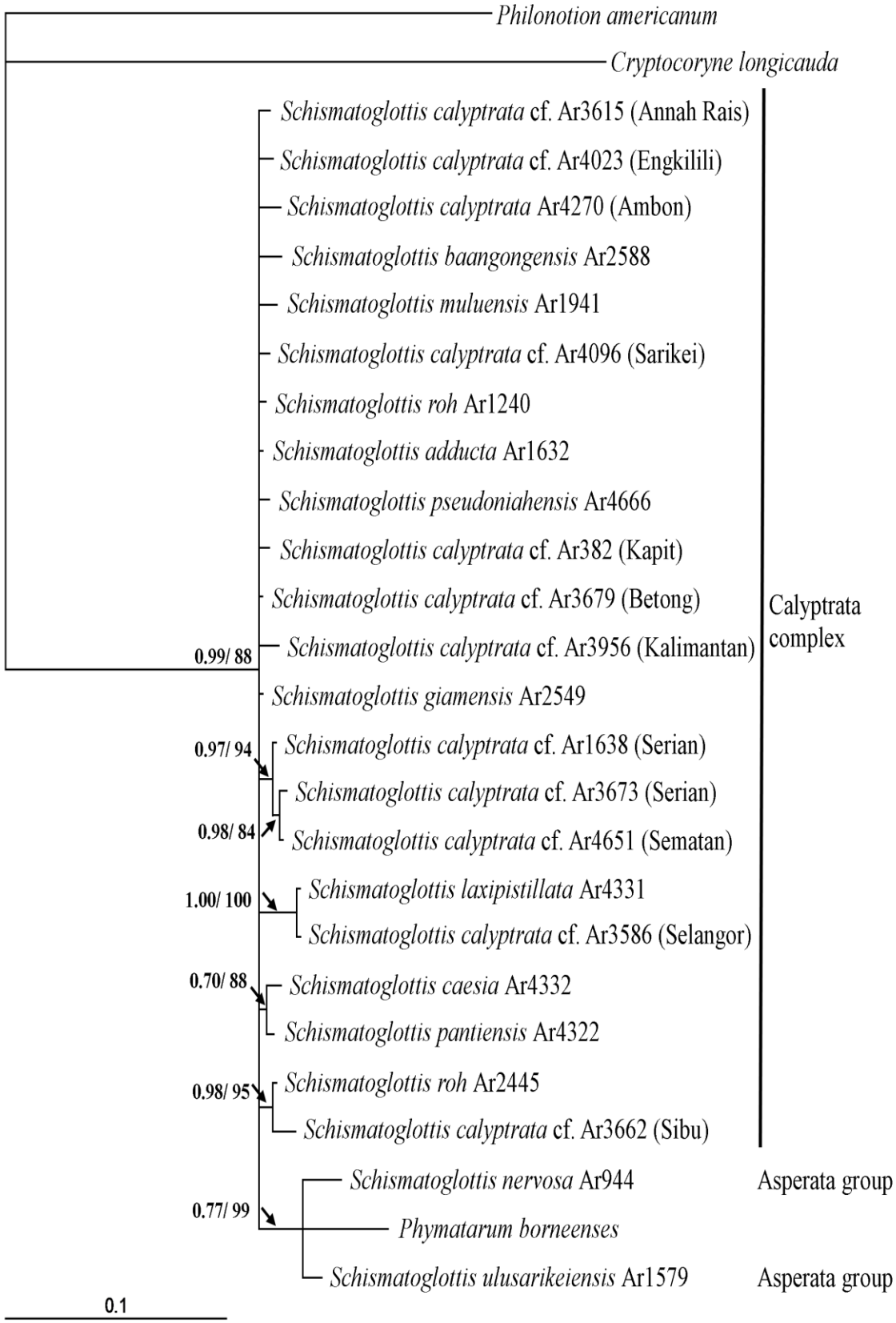

Figure 5.2. Bayesian 50 % majority rule consensus tree obtained using the ITS region. Bayesian posterior probabilities > 0.50 and bootstrap values > 50 % (maximum likelihood) are shown below the branches.

5.3.3 Mapping

The ten taxa (eleven accessions) phylogenetic tree (*mat*K) was selected and mapped with 11 characters (locality, insect visitors, floral scent and morphology) are presented as below (**Figure 5.3**).

5.3.3.1 Locality

The species of Calyptrata complex is widespread, distributed from tropical southwestern China, extending east to New Guinea and Vanuatu (Hay & Yuzammi, 2000). Due to sharp falling off of diversity of genus *Schismatoglottis* in east Borneo, this genus is suggested extended to east of Wallacea (Hay & Yuzammi, 2000). The ten taxa (eleven accessions) phylogenetic tree showed the species of Calyptrata complex comprises two clusters: Peninsular Malaysia – Sarawak and Sarawak – Ambon. Apparently, the species of Calyptrata complex in Peninsular Malaysia (*S. laxipistillata, S. caesia* and *S. pantiensis*) clade with the the species of Calyptrata complex in Sarawak (*S. roh* Ar2445 and *S. giamensis*), not *S. calyptrata* Ar4270 from Ambon. This was probably owing to species of Calyptrata complex from Peninsular Malaysia and Sarawak are confined within the boundaries of Sundaland (Myers *et al.,* 2000), west of Wallace's line. During Pleistocene, glaciation and the lowering sea levels connected the Indonesian archipelago (Sumatra, Java and Borneo) and Indochina (include Peninsular Malaysia) (Voris, 2000). The species of Calyptrata complex probably disperse within Peninsular Malaysia and Sarawak.

Ambon Island is located in south of the western end of the islands of Seram (Strack, 1993), situated within the boundaries of Wallacea, east of Wallace's line (Myres *et al.,* 2000). *Schismatoglottis calyptrata* Ar4270 from Ambon grouped with the remaining species of

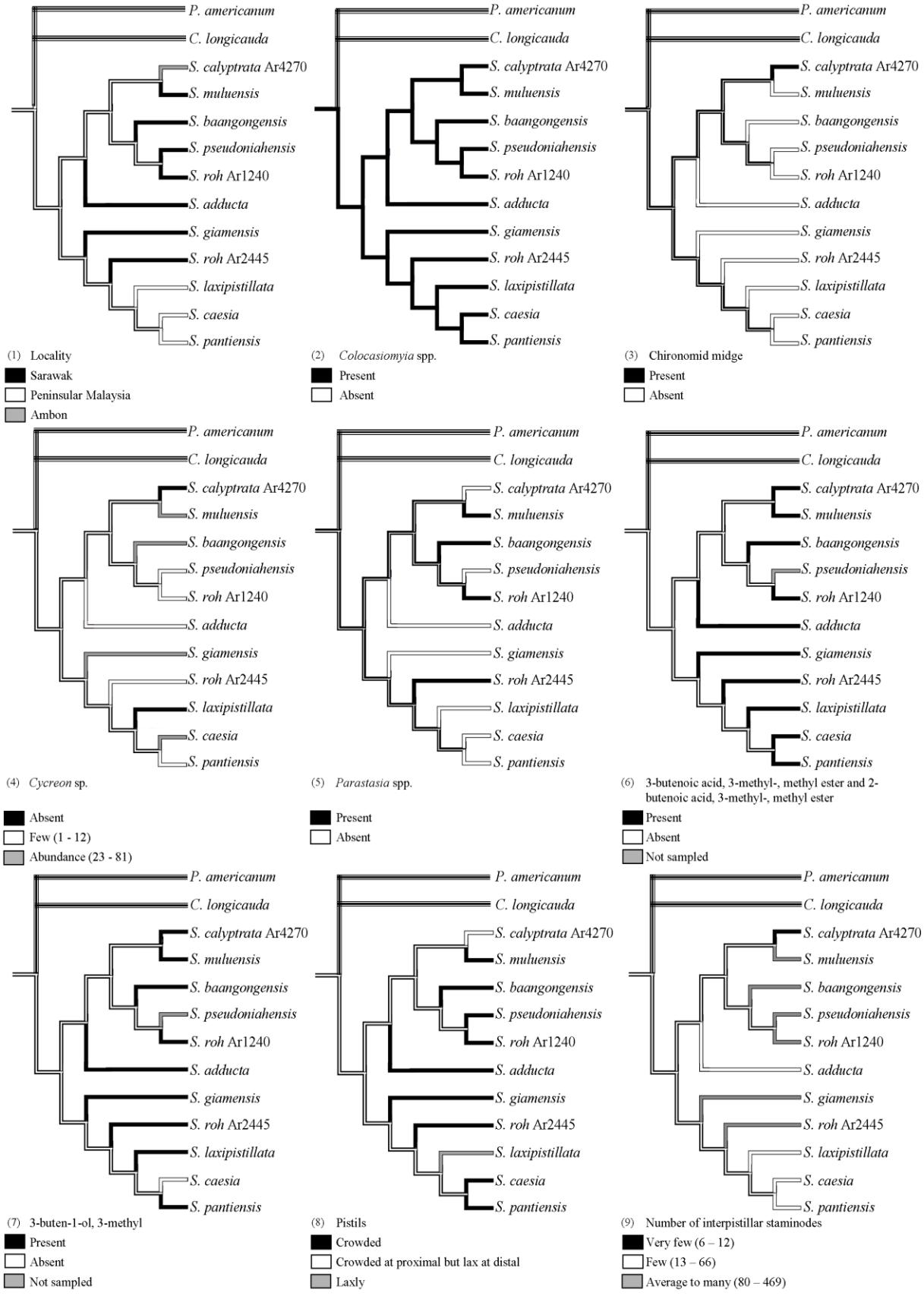

Figure 5.3. Locality, insect visitors, floral scent and morphological characters were mapped onto phylogeny of ten investigated species of the Calyptrata complex.

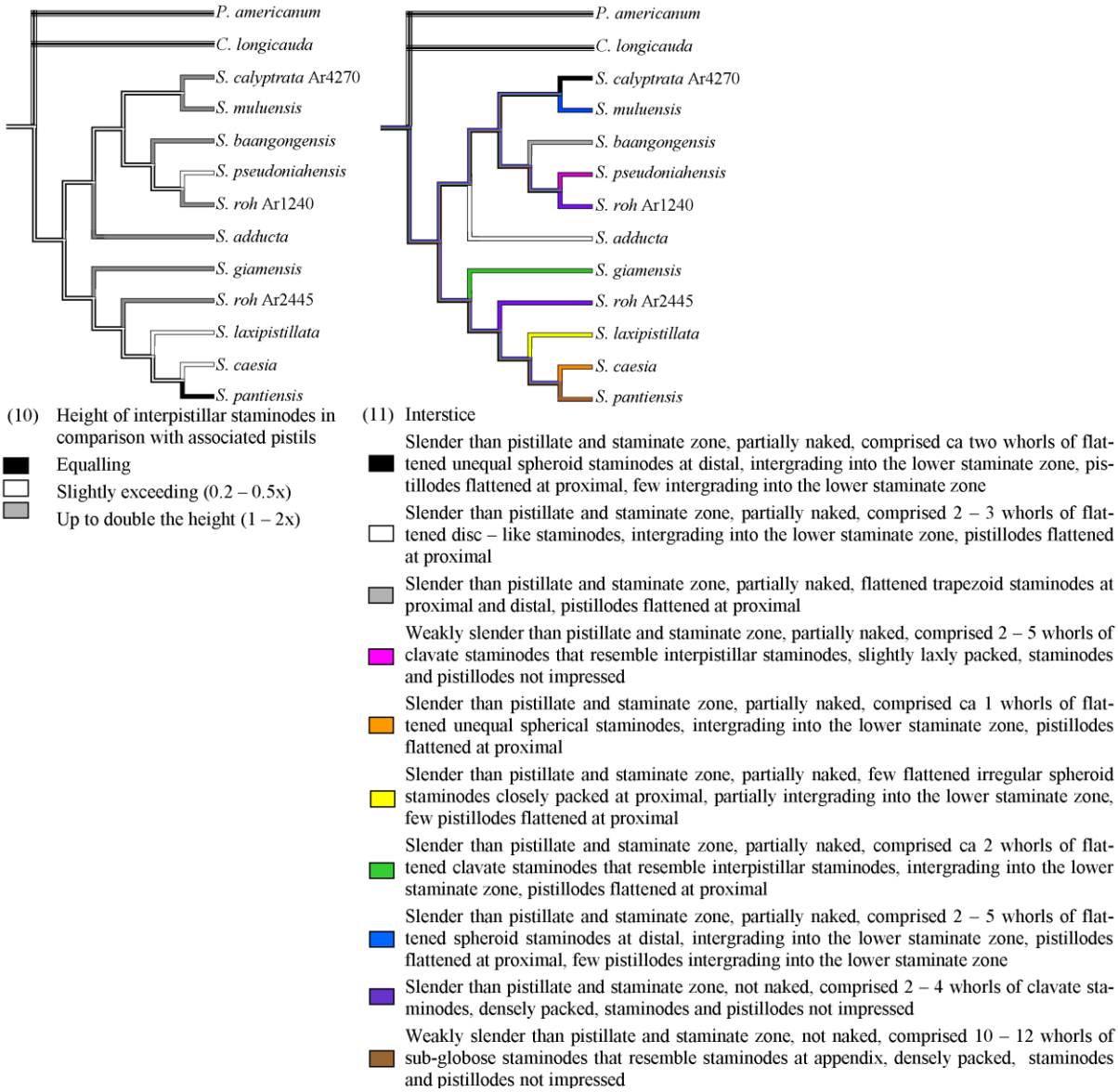

Figure 5.3. Locality, insect visitors, floral scent and morphological characters were mapped onto phylogeny of ten investigated species of the Calyptrata complex.

Calyptrata complex from Sarawak (*S. muluensis, S. baangongensis, S. pseudoniahensis, S. roh* AR1240 and *S. adducta*). During or preceding Miocene time (Linthout & Helmers, 1994), Ambon was faulted off of New Guinea, move westward and northward. Further investigations in more study localities are required especially in New Guinea, Sumatera and Philippines that seemingly do not have low species diversity of *Schismatoglottis* (Hay & Yuzammi, 2000).

5.3.3.2 *Colocasiomyia* spp. (Drosophilidae: Diptera)

In this study, *Colocasiomyia* flies are the pollinators in all the ten investigated taxa of Calyptrata complex. The presence of *Colocasiomyia* flies is plesiomorphic, with all the ten investigated taxa of Calyptrata complex share the similar ancestral character – *Colocasiomyia* pollinator. So far *Colocasiomyia* flies were only reported as the sole pollinator or visitor (Sultana *et al.,* 2006; Toda & Lakim, 2011) in *Schismatoglottis*. These flies visited *S. calyptrata* in Java (Sultana *et al.,* 2006) and Sabah (Toda & Lakim, 2011), *S. wongii* A.Hay and *S. corneri* A.Hay in Sabah (Toda & Lakim, 2011) and *Schismatoglottis* spp. in Java, Sulawesi, Sabah and West Kalimantan (Sultana *et al.,* 2006). In this study, *Colocasiomyia* flies were the main pollinator as these flies were not sporadic, feeding behaviour was not destructive, visited the pistils during pistillate anthesis, left with adhered pollen, and has the highest visitation number among the insect taxa. However, other insect taxa were observed to seek for food rewards (*Cycreon* beetles, *Chaloenus* beetles, *Parastasia* beetles, *Atheta* beetles) and reproductive activities (*Cycreon* beetles, *Chaloenus* beetles, *Parastasia* beetles, *Atheta* beetles, chironomid flies, pteromalid wasps and *Trigona* bees). Recent pollination studies in tribe Schismatoglottideae reported *Colocasiomyia* flies as the main pollinator, and revealed also other insect visitor (*Chaloenus* beetles, *Altica cyanae* beetles and *Cycreon* beetles) (Low *et al.,* 2013; Wong & Boyce, 2014; Low *et al.,* 2015).

The inflorescences in Araceae are commonly known to be pollinated or visited by flies or beetles (Gibernau, 2003 & 2011). Bröderbauer *et al.,* (2012) found the pollination trap in Araceae is correlated with pollination with flies rather than beetles. Although present study revealed various visited insect taxa, it is presumed that *Colocasiomyia* flies are the primitive pollinator for species of Calyptrata complex. Species of Calyptrata complex have no 'constricted' pollination trap and changes of pollination trap from nontraps to traps was happened within fly-pollinated clades, but not associated with simultaneous change in other pollinator type (Bröderbauer *et al.,* 2012).

5.3.3.3 Chironomidae Midge (Diptera)

The presence of chironomid midge is apomorphic in *S. calyptrata* Ar4270. All species of Calyptrata complex were not visited by the chironomid midge except *S. calyptrata* Ar4270 in Ambon. Chironomid midge is considered as the visitor as it was observed only laid eggs on staminate flower zone and left prior to inter-anthesis. So far, no study report chironomid midge visits the aroid inflorescence in tropic (Gibernau, 2003 & 2011). However, chironomid midges (*Pyschoda phalaenoides* L. and *Smittia pratorum* Goetghebuer) were pollinator that carried pollen of *Arum maculatum* L. and *A. italicum* Miller (Diaz & Kite, 2002) in Southern England. Other studies reported *P. phalaenoides* were the pollinator of *A. maculatum* (Lack & Diaz, 1991; Kite, 1995).

5.3.3.4 *Cycreon* sp. (Hydrophilidae: Coleoptera)

All species of Calyptrata complex were visited by the nitidulid *Cycreon* beetle except *S. calyptrata* Ar4270 and *S. laxipistillata* Ar4332. Presence of *Cycreon* beetles is plesiomorphic in all species of Calyptrata complex in Sarawak. The Calyptrata species in Sarawak share this

similar ancestral character (visited by *Cycreon* beetles). Recent pollination studies in Sarawak reported unidentified nitidulid beetle visited all seven investigated *Homalomena* spp. except *H. velutipedunculata* S.Y.Wong, Y.C.Hoe & P.C.Boyce. Within the species of Hanneae Complex, *H. velutipedunculata* is distinctive by its velvety peduncle minutely puberulent (Wong *et al.,* 2013) and has *sec*-butyl acetate and monoterpenes floral VOCs (Hoe *et al.,* 2016).

Studies recorded *Cycreon* beetles as the visitor in *P. borneense* M.Hotta, *S. sarikeense* (Bogner & M.Hotta) P.C.Boyce & S.Y.Wong and *Homalomena* spp. (Low *et al.,* 2015; Hoe *et al.,* 2016). In *P. borneense* and *S. sarikeense,* the visitation number of *Cycreon* beetles visitor were low (0 – 7) (Low *et al.,* 2015). In *Homalomena* spp., two patterns of visitation number were observed: abundance number (22 – 50) of *Cycreon* beetles presented in *H. baangongensis,* where else few numbers (0 – 17) of *Cycreon* beetles presented in other six *Homalomena* spp. (Hoe *et al.,* 2016). In this study, *Cycreon* beetles also have two patterns of visitation number. Abundance number (23 – 81) of *Cycreon* beetles visited *S. muluensis, S. baangongensis, S. giamensis* and *S. caesia.* These beetles were not sporadic, left with adhered pollen. Few numbers (1 – 12) of *Cycreon* beetles visited *S. pseudoniahensis, S. roh* Ar1240, *S. adducta, S. roh* Ar2445 and *S. pantiensis.* These beetles sometime sporadic, and left with adhered pollen (*S. adducta, S. roh* Ar1240 and *S. pantiensis*).

5.3.3.5 *Parastasia* spp. (Scarabaeidae: Coleoptera)

The latest revision in *Parastasia* beetles recorded *Parastasia* beetles present in Peninsular Malaysia, Ambon and Sarawak (Kuijten, 1992). However, in present study, *Parastasia* beetles were only found in most species of Calyptrata complex in Sarawak (*S. baangongensis,*

S. muluensis, S. roh Ar2445*, S. roh* Ar1240). So far, association of *Parastasia* pollinator only reported in *Homalomena* spp. in Sarawak (Momose *et al.,* 1998; Kumano & Yamaoka, 2006; Kumano-Nomura & Yamaoka, 2009; Tung *et al.,* 2010; Hoe *et al.,* 2011; Hoe *et al.,* 2012; Hoe *et al.,* 2016).

Parastasia beetles visited *S. muluensis, S. baangongensis, S. roh* Ar1240 and *S. roh* Ar2445 that bear moderate to many (80 – 469) number of interpistillar staminodes. Coincidentally, *S. roh* Ar1240 bear the highest number of interpistillar staminodes (362 – 469) visited by the highest number of *Parastasia* beetles (4 ± 5) (**Table 4.1**). Foraging behaviour of *Parastasia* beetles was fully observed in *S. roh* Ar1240 and *S. roh* Ar2445 and these beetles consumed the interpistillar staminodes. Where else *Parastasia* beetle only sampled from bagged inflorescences of *S. baangongensis* and *S. muluensis* that bear moderate number of interpistillar staminodes (86 – 158). Probably *Parastasia* beetles are more sporadic in *S. baangongensis* and *S. muluensis.* However, no *Parastasia* beetle present for species of Calyptrata complex in Ambon and Peninsular Malaysia that bearing very few to few number of interpistillar staminodes. Further studies are required to strengthen present of *Parastasia* beetles are associated with studied locality or the number of interpistilar staminodes on the inflorescence.

5.3.3.6 3-Butenoic acid, 3-methyl-, methyl ester and 2-butenoic acid, 3-methyl-, methyl ester

All species of Calyptrata complex emitted dominant floral VOC of 3-butenoic acid, 3-methyl-, methyl ester (96.35 – 99.89%) and minor VOC of 2-butenoic acid, 3-methyl-, methyl ester (0.09 – 0.72%). The presence of 3-butenoic acid, 3-methyl-, methyl ester and 2-butenoic acid,

3-methyl-, methyl ester are plesiomorphic in all species of Calyptrata complex (except floral scent of *S. pseudoniahensis* is not sampled). To date, 3-butenoic acid, 3-methyl-, methyl ester only recorded as minor VOC (0.06%) in the petals of *Robinia pseudoacacia* L. (Fabaceae) (Aronne *et al.,* 2014). Where else 2-Butenoic acid, 3-methyl-, methyl ester was recorded in several angiosperm families: Cycadaceae (Pellmyr *et al.,* 1991), Asteraceae (Pham-Delegue *et al.,* 1989), Amaryllidaceae (Dobson *et al.,* 1997) and Arecaceae (Kundsen, 1999). The role of these compounds are not further discuss except Dobson *et al.,* (1997) recognized the phenetic grouping of *Narcissus* spp. by based on floral scent data. Among the nine investigated species of *Narcissus* spp., *N. assoanus* Dufour and *N. jonquilla* L. that emitted 2-butenoic acid, 3-methyl-, methyl ester and other isopentenoid ester and benzenoid ester formed into a clade itself. Where else *N. cuatrecasasii* Fern.Casas (fatty-acid dominance), *N. bulbocodium* L. and *N. triandrus* L. (monoterpenes dominance) formed into a separated clade themselves. Coincidentally, *N. assoanus* and *N. jonquilla* also share the similar insect visitor of Sphingidae (Lepidoptera) moth (Dobson *et al.,* 1997). Studies in *Homalomena* spp. showed the species of Giamensis complex shared dominance VOCs (*sec*-butyl acetate, (*E*)- and (*Z*)-4,8-dimethyl-1,3,7-nonatriene) pollinated by *Parastasia* spp. (Hoe e*t al.,* 2016). In this study, all species of Calyptrata complex emitted 3-butenoic acid, 3-methyl-, methyl ester and 2-butenoic acid, 3-methyl-, methyl ester, pollinated by *Colocasiomyia* flies. This implies 3-butenoic acid, 3-methyl-, methyl ester and 2-butenoic acid, 3-methyl-, methyl ester may play an importance role in attracting the similar *Colocasiomyia* pollinator.

5.3.3.7 3-Buten-1-ol, 3-methyl-

The presence of 3-buten-1-ol, 3-methyl- is plesiomorphic in all species of Calyptrata complex except this compound is absent in *S. caesia* (floral scent of *S. pseudoniahensis* is not sampled).

The absent of 3-buten-1-ol, 3-methyl- is apomorphic in *S. caesia* as this specialized character is only found in *S. caesia.* 3-buten-1-ol, 3-methyl- was detected as minor VOC in *Parkia biglobosa* Jacq. (Leguminosae: Mimosoideae) (< 0.71%) (Petersson & Kundsen, 2001), *Lathyrus odoratus* L. (< 0.3%) (Porter *et al.,* 1999) and *Narcissus* spp. (Amaryllidaceae) (Dobson *et al.,* 1997). The role of this compound is not further discussed except phenetic classification in *Narcissus* spp. by Dobson *et al.* (1997). Among the investigated *Narcissus* spp., 3-buten-1-ol, 3-methyl- presented in *N. assoanus* (0.6%), *N. jonquilla* (< 0.8%) and *N. bulbocodium* (< 7%). *Narcissus assoanus* and *N. jonquilla* that emitted 3-buten-1-ol, 3-methyl- and isopentenoid ester compounds were clade together. However, *N. bulbocodium* that emitted 3-buten-1-ol, 3-methyl- but lacking isopentenoid ester compounds and comprised mainly myrcene is distinguished by clade with *N. triandrus* (mainly monoterpenes) and *N. cuatrecasasii* (fatty acid derived acetates). In present study, *S. caesia* is distinguished by did not emitted 3-buten-1-ol, 3-methyl- and is apomorphic within the species of Calytrata complex in Peninsular Malaysia.

5.3.3.8 Pistils

Previous studies identified four types of arrangement of pistils in species of Calyptrata Group: 1) crowded, 2) crowded at proximal and laxly at distal, 3) laxly and 4) pistils laxly and distal more very laxly (Hay & Yuzammi, 2000; Scherberich & Boyce, 2013, Wong, 2012). The pistils crowded on pistillate flower zone is identified in most species of Calyptrata Group (*S. scintillans* Scherberich & P.C.Boyce*, S. ranchanensis* S.Y.Wong*, S. heterodoxa* S.Y.Wong*, S. zonata* Hallier f.*, S. wongii* A.Hay*, S. wahaiana* Alderw.*, S. unifolia* A.Hay & P.C.Boyce*, S. trusmadiensis* A.Hay & J.Mood*, S. trivittata* Hallier f., *S. pumila* Hallier f.ex Engl., *S. plurivenia* Alderw*, S. niahensis* A.Hay*, S. moodii* A.Hay*, S. edanoi* A.Hay, sp. nov.*, S.*

ecaudata A.Hay*, S. decipiens* A.Hay*, S. clarae* A.Hay*, S. canaliculata* Engl., *S. bogneri* A.Hay), subsequently pistils crowded in the proximal and laxly in the distal (*S. subundulata* (Zoll.ex Schott) Nicolson, *S. pectinervia* A.Hay*, S. modesta* Schott*, S. lingua* A.Hay, *S. bifasciata* Engl. and *S. ahmadii* A.Hay), pistils laxly (*S. gamoandra* M.Hotta*, S. viridissima* A.Hay and *S. silamensis* A.Hay) and pistils laxly and distal more very laxly (*S. viridissima* and *S. venusta* A.Hay). The first three types of above mentioned arrangement of pistils are identified in investigated species of Calyptrata complex. Pistils crowded shared by most of the species (*S. muluensis, S. baangongensis, S. pseudoniahensis, S. roh* Ar1240, *S. adducta, S. giamensis, S. roh* Ar2445, *S. caesia* and *S. pantiensis*) and are plesiomorphic in all species of Calyptrata complex in Sarawak. Pistils crowded in the proximal and laxly in the distal is found in *S. calyptrata* Ar4270, similar with description in Hay & Yuzammi (2000) (pistils closey packed, distally becoming more widely spaced). Pistils laxly on the entire pistillate flower zone are found only in *S. laxipistillata.*

5.3.3.9 Number of Interpistillar Staminodes

Previously studies recorded the number of interpistillar staminodes in species of Calyptrata Group are none, very few or none, few, present and very numerous (Hay & Yuzammi, 2000; Wong, 2012; Scherberich & Boyce, 2013). In this study, all species of Calyptrata complex bear interpistillar staminodes. Different mean number of interpistillar staminodes in species of Calyptrata complex probably associated with the 'localities' and 'visited insects'. In Ambon, *S. calyptrata* Ar4270 can be differentiated by bearing very few number of interpistillar staminodes (6 – 12). Very few number of interpistillar staminodes in *S calyptrata* Ar4270 is presumably associates with its non-destructive visited insects that did not chew the interpistillar staminodes and absent of beetle.

All species of Calyptrata complex in Peninsular Malaysia: *S. laxipistillata, S. caesia* and *S. pantiensis* formed a clade themselves and bearing few numbers of interpistillar staminodes (13 – 66). The numbers of interpistillar staminodes of *S. laxipistillata, S. caesia* and *S. pantiensis* are slightly many than *S. calyptrata* Ar4270 in Ambon (6 – 12) is probably the destructive *Chaloenus* beetles that consumed the interpistillar staminodes presented in all species of Calyptrata complex in Peninsular Malaysia.

All the species of Calyptrata complex In Sarawak bear average to many number of interpistillar staminodes (80 – 469) except *S. adducta* bear few number of interpistillar staminodes. The species of Calyptrata complex in Sarawak have more number of interpistillar staminodes than species of Calyptrata complex in Peninsular Malaysia and Ambon. This probably owing to more destructive insects (*Chaloenus* spp. and *Parastasia* spp.) visited the species of Calyptrata complex in Sarawak, notably *S. muluensis, S. baangongensis, S. roh* Ar2445 and *S. roh* Ar1240 visited by both *Chaloenus* spp. and *Parastasia* spp.. Among *S. muluensis, S. baangongensis, S. roh* Ar2445 and *S. roh* Ar1240, coincidentally, *S. roh* Ar1240 has the highest number of interpistillar staminodes (up to 469) visited by the highest number of *Parastasia* beetles (up to four individuals). Where else *S. muluensis, S. baangongensis* and *S. roh* Ar2445 have 150 – 170 number of interpistillar staminodes only visited by 1 – 2 *Parastasia* beetles. Previous studies reported the protein and carbohydrate rich interpistillar staminodes (*Dieffenbachia longispatha* Engl.) was the food reward for scarab beetles (*Cyclocephala gravis*) (Young, 1986). In Sarawak, *Parastasia* scarab beetles and chrysomelids beetles (*Chaloenus* spp. and *Dercetina* sp.) consumed the interpistillar staminodes of *Homalomena* spp. (Kumano & Yamaoka, 2009; Tung *et al.,* 2000; Hoe *et al.,* 2011 & 2016). Phylogeny study showed *Homalomena* Cyrtocladon Supergroup species that

bear interpistillar staminodes visited by *Chaloenus* beetles and *Parastasia* beetles. Where else absent of interpistillar staminodes in Punctulata Supergroup and *H. expedita* (Homalomena Supergroup) implies another alternative pollination mechanism, and *H. expedita* only visited by *Colocasiomyia* flies (Wong *et al.*, 2013).

5.3.3.10 Height of Interpistillar Staminodes in Comparison with Associated Pistils

Previous studies identified various types of 'height of interpistillar staminodes in comparison with associated pistils' in species of Calyptrata Group, which include 1) interpistillar staminodes shorter than the pistils (*S. silamensis*), 2) shorter than to subequalling (*S. longispatha* W. Bull), 3) equalling (*S. bogneri, S. ecaudata, S. pumila, S. trusmadiensis, S. unifolia, S. venusta, S. viridissima*), 4) equalling to slightly taller (*S. ahmadii, S. eymae* A.Hay), 5) slightly taller to taller than (*S. longifolia* Ridl*., S. modesta, S. trivittata, S. motleyana* (Schott) Engl*., S. scintillans, S. edanoi, S. heterodoxa, S. ranchanensis*), 6) 1.5x the height of the pistils (*S. bifasciata, S. niahensis*) and 7) 2x the height of the pistils (*S. canaliculata, S. decipiens, S. lingua, S. moodii, S. trifasciata* Engl*., S. wallichii* Hook.f.) (Hay & Yuzammi, 2000; Scherberich & Boyce, 2013; Wong, 2012). Among the species of Calyptrata complex, interpistillar staminodes equaling with associated pistils present in *S. pantiensis*, slightly exceeding (0.2x – 0.5x the height of pistils) is found in *S. pseudoniahensis, S. laxipistillata* and *S. caesia*, up to double (1x – 2x the height of pistils) present in most species of Calyptrata complex: *S. calyptrata* Ar4270, *S. muluensis, S. baangongensis, S. roh* Ar1240*, S. adducta, S. giamensis* and *S. roh* Ar2445.

Interpistillar staminodes up to double the height of pistils are plesiomorphic in species of Calyptrata complex in Sarawak, except *S. pseudoniahensis.* Coincidentally, most species of

Calyptrata complex in Sarawak visited by *Parastasia* beetles that consumed the interpistillar staminodes. The longest stalk of interpistillar staminodes in species of Calyptrata complex in Sarawak may indicate an easier access of food reward (chewing) for *Chaloenus* beetles and *Parastasia* beetles. *Schismatoglottis pseudoniahensis* does not has interpistillar staminodes up to double the height of pistils probably due to this species did not visited by *Chaloenus* beetles and *Parastasia* beetles. The species of Calyptrata complex in Peninsular Malaysia have only equaling to slightly exceeding interpistillar staminodes may due to these species only visited by *Chaloenus* beetles, but not *Parastasia* beetles. The *S. calyptrata* Ar4270 in Ambon did not visited by any destructive beetle but has interpistillar staminodes up to double the height of pistils. It is presume that *S. calyptrata* Ar4270 reduce its number of interpistillar staminodes (6 – 12) instead of the height of the interpistillar staminodes.

5.3.3.11 Interstice

The interstice among the species of Calyptrata Group species is variable. Interstice is absent in *S. wongii*, *S. motleyana*, *S. canaliculata*, *S. bifasciata, S. trifasciata* Complex, *S. unifolia* (Hay & Yuzammi, 2000) and *S. scintillans* (Scherberich & Boyce, 2013). However, interstice is present in all investigated species of Calyptrata complex. Interstice is slightly and abruptly thicker than upper pistillate flower zone in *S. eymae* and *S. gamoandra* (Hay & Yuzammi, 2000). In this study, interstice is found slender than pistillate and staminate flower zone (*S. baangongensis, S. giamensis, S. roh* Ar1240, *S. roh* Ar2445, *S. adducta, S. laxipistillata, S. ceasia, S. calyptrata* Ar4270 and *S. muluensis*) or weakly slender than pistillate and staminate flower zone (*S. pantiensis* and *S. pseudoniahensis*).

Among the investigated species of Calyptrata complex, only staminodes and pistillodes at interstice of *S. pantiensis, S. pseudoniahensis, S. roh* Ar1240 and *S. roh* Ar2445 do not impressed. *Schismatoglottis pseudoniahensis* can be differentiated by interstice partially naked, comprised 2 – 5 whorls of clavate staminodes that resemble interpistillar staminodes, slightly laxly packed. *Schismatoglottis pantiensis*, *S. roh* Ar1240 and *S. roh* Ar2445 interstice is not naked and staminodes densely packed. However, *S. pantiensis* can be differentiated by comprised 10 – 12 whorls of sub-globose staminodes that resemble staminodes at appendix, where else *S. roh* Ar1240 and *S. roh* Ar2445 comprised 2 – 4 whorls of clavate staminodes. Interstice of *S. ranchanensis* (Wong, 2012) is rather similar with *S. pantiensis* where the staminodes resemble staminodes of appendix. However, the former has longer interstice (ca ¼ of spadix length) than the latter (ca $\frac{1}{7}$ of spadix length).

Partially naked interstice is present in several species of Calyptrata Group (*S. ahmadii*, *S. trivittata*, *S. trusmadiensis* and *S. venusta*) (Hay & Yuzammi, 2000) and subsequent investigated species of Calyptrata complex. All subsequent investigated species of Calyptrata complex has staminodes intergrading into the lower staminate flower zone except *S. laxipistillata* and *S. baangongensis*. *Schismatoglottis laxipistillata* is distinctive by interstice has few flattened irregular spheroid staminodes closely packed at proximal, partially intergrading into the lower staminate flower zone, where else *S. baangongensis* has flattened trapezoid staminodes at proximal and distal, staminodes do not intergrading into the lower staminate flower zone. Abortive ovaries intergrading into the lower staminate flower zone present in *S. venusta* (Calyptrata Group) (Hay & Yuzammi, 2000). However, pistillodes intergrading into the lower staminate flower zone are only found in *S. calyptrata* Ar4270 and *S. muluensis*. *Schismatoglottis calyptrata* Ar4270 can be distinguished by interstice comprised

ca two whorls of flattened unequal spheroid staminodes at distal, where else *S. muluensis* comprised 2 – 5 whorls of flattened spheroid staminodes at distal. The following species, *S. giamensis* can be differentiated by interstice comprised ca 2 whorls of flattened clavate staminodes that resemble interpistillar staminodes (flattened). *Schismatoglottis adducta* can be distinguished by having 2 – 3 whorls of flattened disc – like staminodes, where else *S. caesia* comprised ca 1 whorls of flattened unequal spheroid staminodes.

CHAPTER 6

CONCLUSIONS AND FUTURE WORK

6.1 Conclusions

This study re-circumscribed *S. calyptrata* (Roxb.) Zoll. & Moritzi, resurrected *Schismatoglottis muluensis* M.Hotta, and revealed eight novel species in *Schismatoglottis* Calyptrata complex. Phylogenetic tree reconstruction using *mat*K region partially resolved the relationship among species in Calyptrata complex. The data from locality, morphological characters, pollinators and floral VOCs were mapped onto the phylogenetic tree and provided further evidence on the species delimitation in Calyptrata complex.

All species of Calyptrata complex are pollinated by *Colocasiomyia* flies, emitted VOCs of 3-butenoic, 3-methyl, methyl ester and 2-butenoic acid, 3-methyl, methyl ester. *Schismatoglottis calyptrata* can be differentiated by the presence of chironomid midges, absence of beetles and has very few number of interpistillar staminodes (6 – 12). Species of Calyptrata complex in Peninsular Malaysia is distinctive by the presence of destructive *Chaloenus* beetles and bearing few number of interpistillar staminodes (13 – 66). Species of Calyptrata complex in Sarawak are visited by *Chaloenus* beetles and *Parastasia* beetles (*S. muluensis, S. baangongensis, S. roh* Ar2445 and *S. roh* Ar1240) and bear average to many interpistillar staminodes (80 – 469).

Pollination investigations revealed *Colocasiomyia* flies as the main pollinator, *Cycreon* beetles as secondary pollinator, *Chaloenus* beetles and *Parastasia* beetles as the opportunistic

pollinator, *Atheta* staphylinid beetles as the visitors (except as pollinator in *S. muluensis*), *Trigona* bees, Pteromalid wasps and Chironomid midges as the visitors. The species of Calyptrata complex in Sarawak were visited by higher insect taxa than species of the Calyptrata complex in Ambon and Peninsular Malaysia. *Parastasia* beetles and *Atheta* beetles were only present in species of Calyptrata complex in Sarawak. These pollinator/ opportunistic pollinator are not specific as other unidentified adhered pollen was found (*Colocasiomyia* flies, *Cycreon* beetles, *Chaloenus* beetle) and *Parastasia* beetles also visited inflorescence of *Homalomena* spp.

Thermogenesis investigations revealed biphasic peak, the first peak occured during pistillate anthesis, the second was during staminate anthesis (*S. adducta, S. calyptrata, S. giamensis, S. pseudoniahensis* and *S. roh* Ar1240). During pistillate anthesis, the larger heat production on appendix and staminate flower zone is presumably to support high energetic activities (courtship, mating and fighting), whereas the lesser heat production on pistillate flower zone supports low energetic activities (eating and sucking the interpistillar staminodes and liquid secreted from the stigma). During staminate anthesis, the appendix generated the highest temperature and *Chaloenus* beetles and *Parastasia* beetles competed for the space on the appendix is presumed to gain more heat reward before departing.

The inflorescence of all species of Calyptrata complex emitted ester compound class, with dominant compound of 3-butenoic acid, 3-methyl-, methyl ester and minor amount of 2-butenoic acid, 3-methyl-, methyl ester. These VOCs are presumed to play the crucial role in pollinator attraction. At period I (0600 – 0800), the appendix emitted highest amount and number of VOCs, following by spathe, staminate flower zone, and pistillate flower zone. This

indicates all different inflorescence parts contribute in pollinator attraction, especially the appendix. At period II (0815 – 1015), only pistillate flower zone increased its total amount of floral scent released. Most insects remain resting in the lower spathe chamber imply more adhered pollen could be transferred onto the pistils.

6.2 Future Work

To further understand evolution relationship among *Schismatoglottis,* further investigations are proposed as below:

- Phylogeny of the insect pollinators and visitors should be carried out to investigate possible correlation with the phylogeny of the *Schismatoglottis*.
- Additional gene regions should be carried out to study the phylogeny of the species of the Calyptrata complex.
- Current investigations in this study to be expanded to other groups and complexes of *Schismatoglottis.*
- *In-situ* floral VOCs synthetic test should be carried out to test the sensory response of the pollinators and visitors towards floral VOCs.
- Field pollination investigations should be carried out at different times of several consecutive years.

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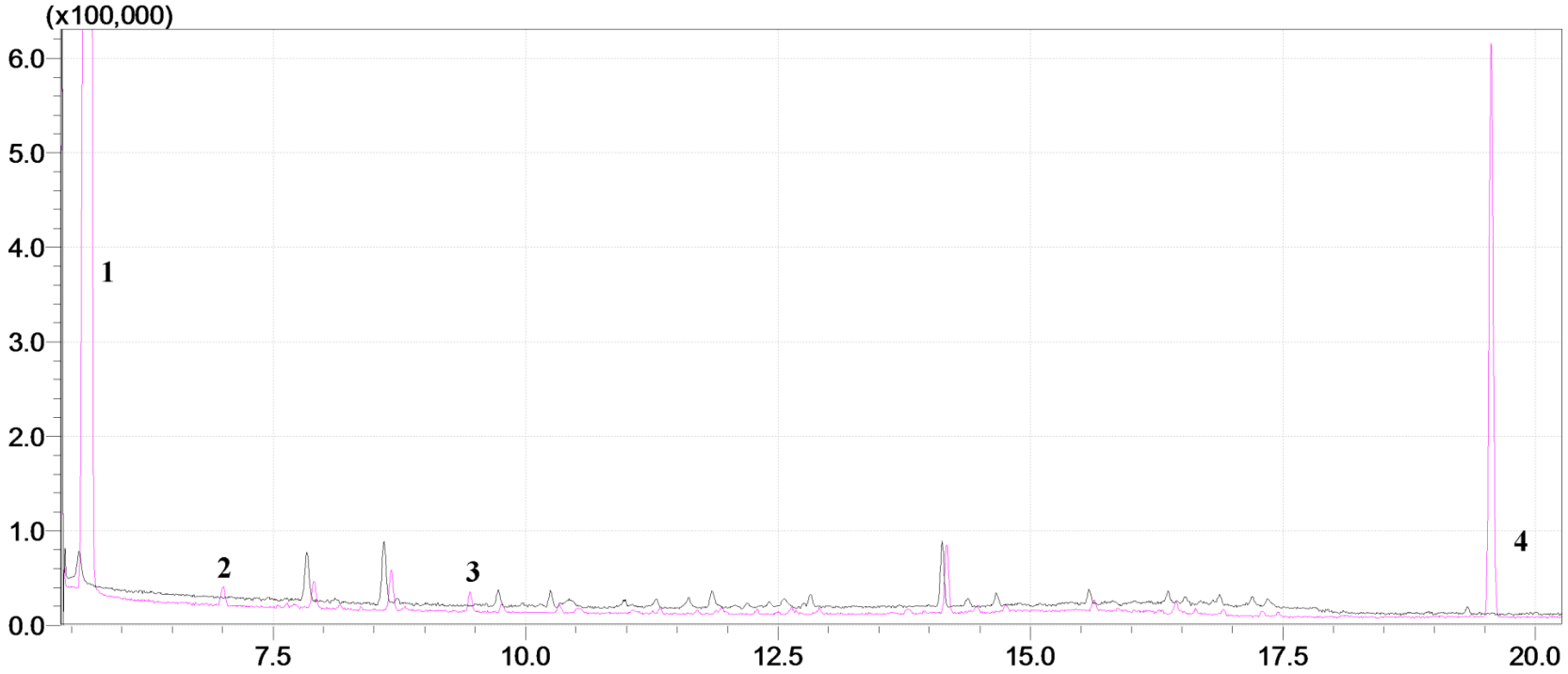
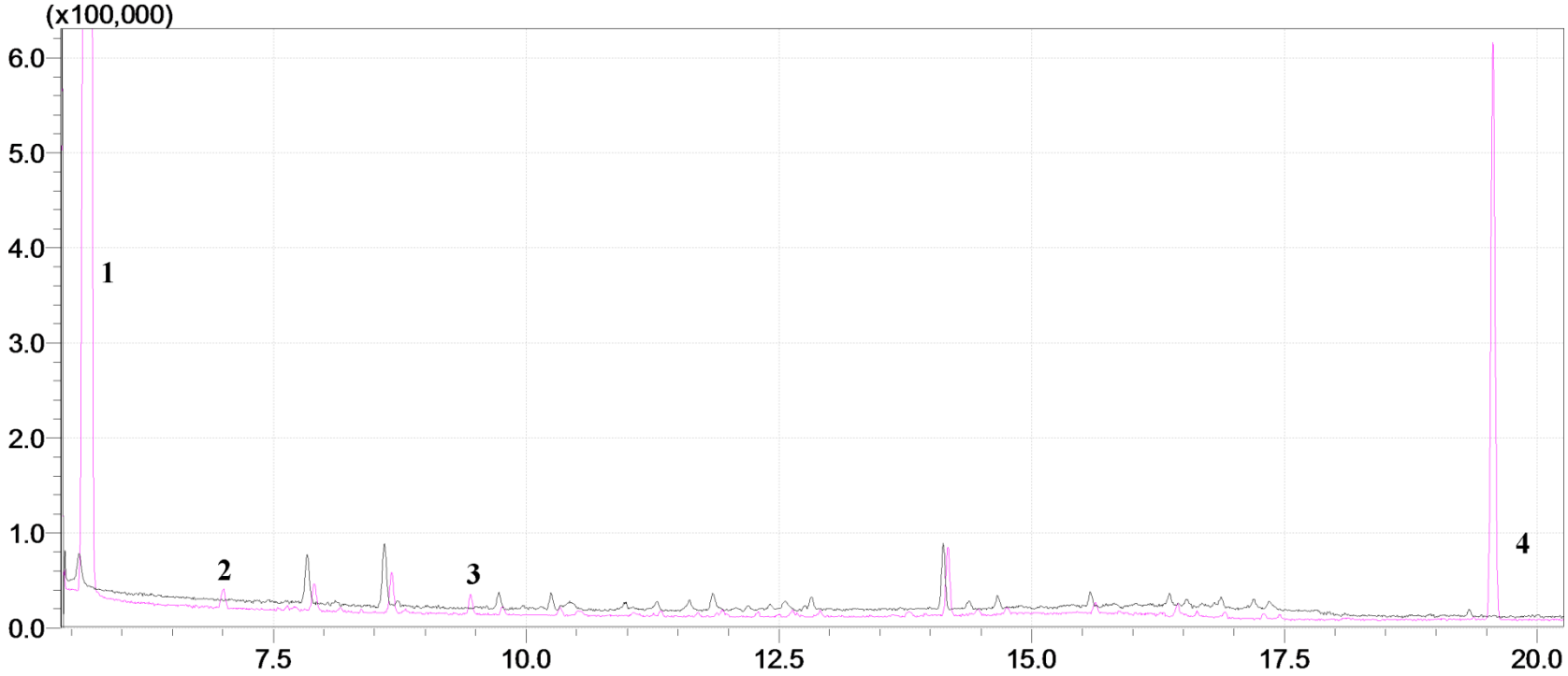
APPENDICES

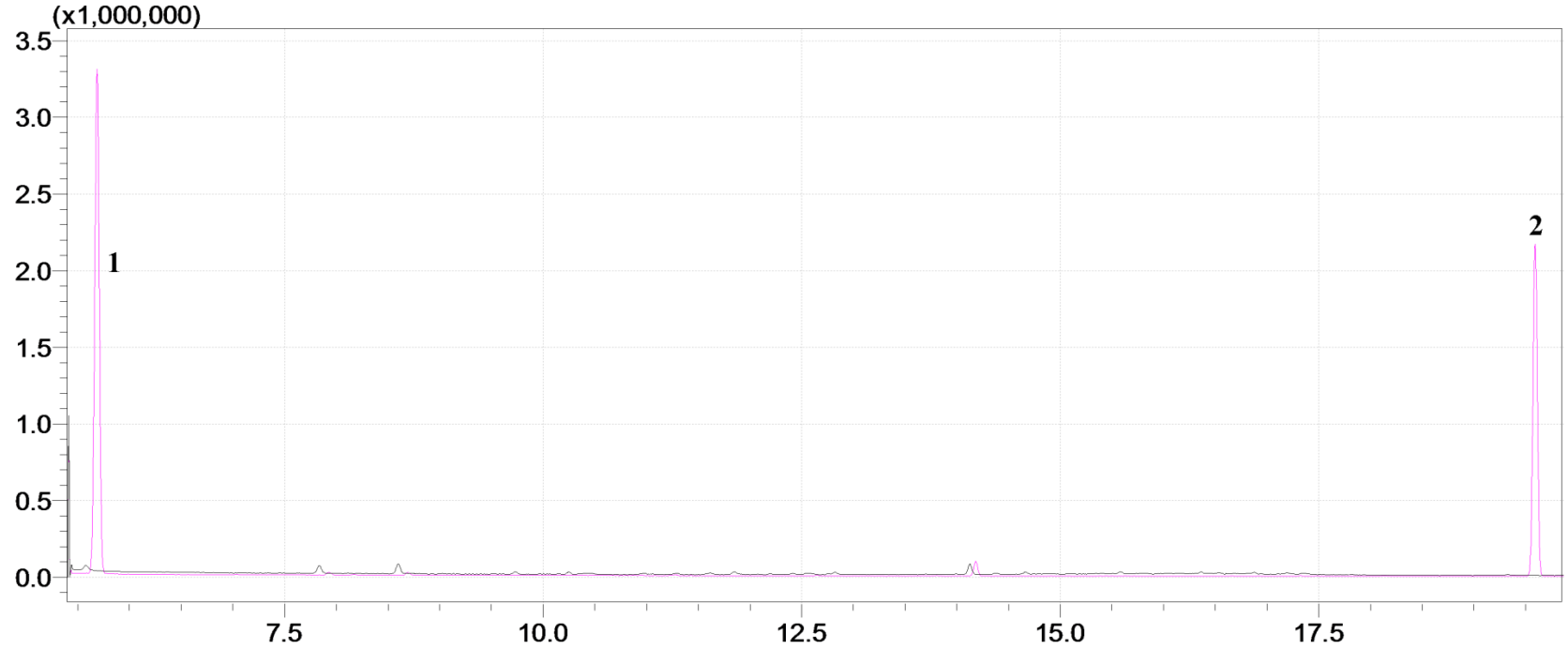
Appendix 1. Representative chromatogram for inflorescence of the species of Calyptrata complex (*S. baangongensis*) that analyzed by BP20 polar column in split mode. 1= 3-Butenoic acid, 3-methyl-, methyl ester; 2= 2-Butenoic acid, 3-methyl-, methyl ester; 3= 3-Buten-1-ol, 3-methyl-; 4= Methyl benzoate.



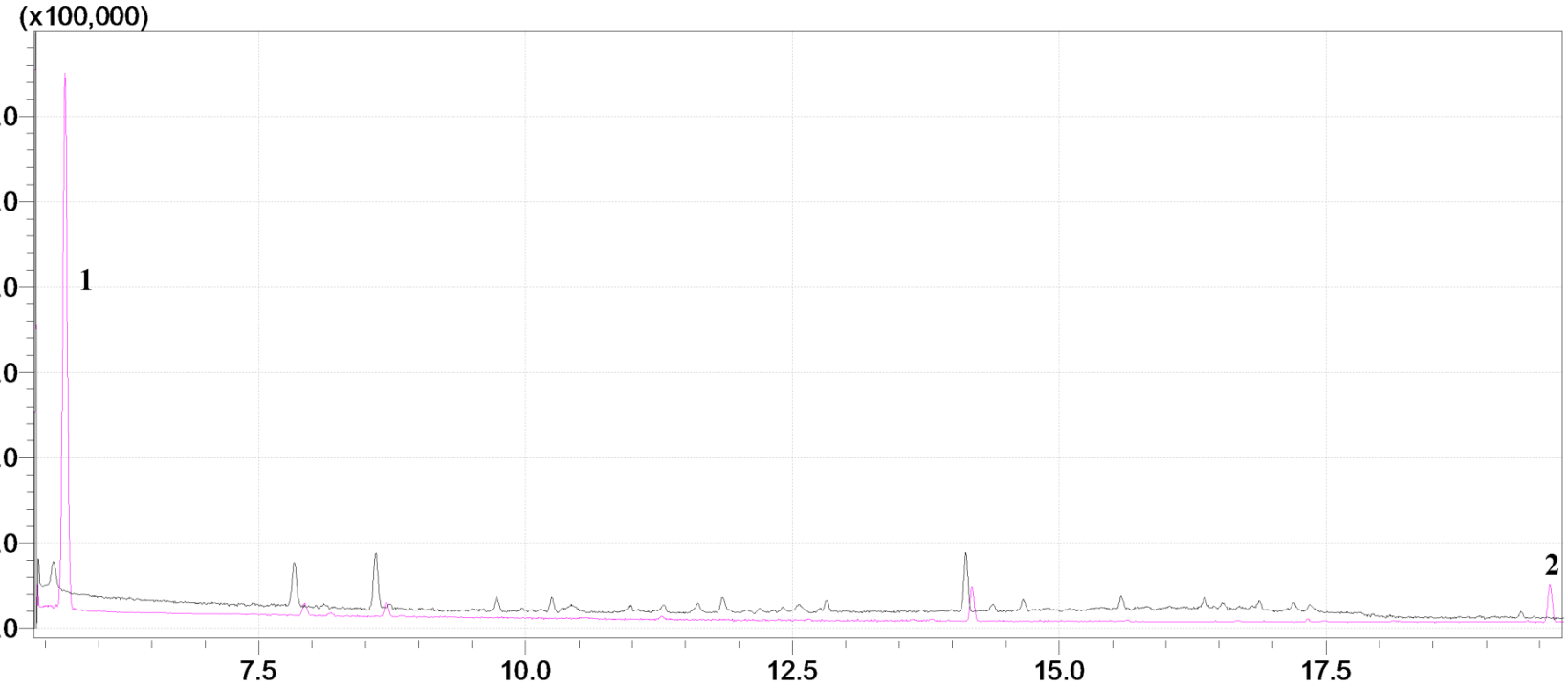
Appendix 2. Representative **c**hromatogram for appendix of the species of Calyptrata complex (*S. baangongensis*) that analyzed by BP20 polar column in split mode. 1= 3-Butenoic acid, 3-methyl-, methyl ester; 2= 2-Butenoic acid, 3-methyl-, methyl ester; 3= 3-Buten-1-ol, 3-methyl-; 4= Methyl benzoate.



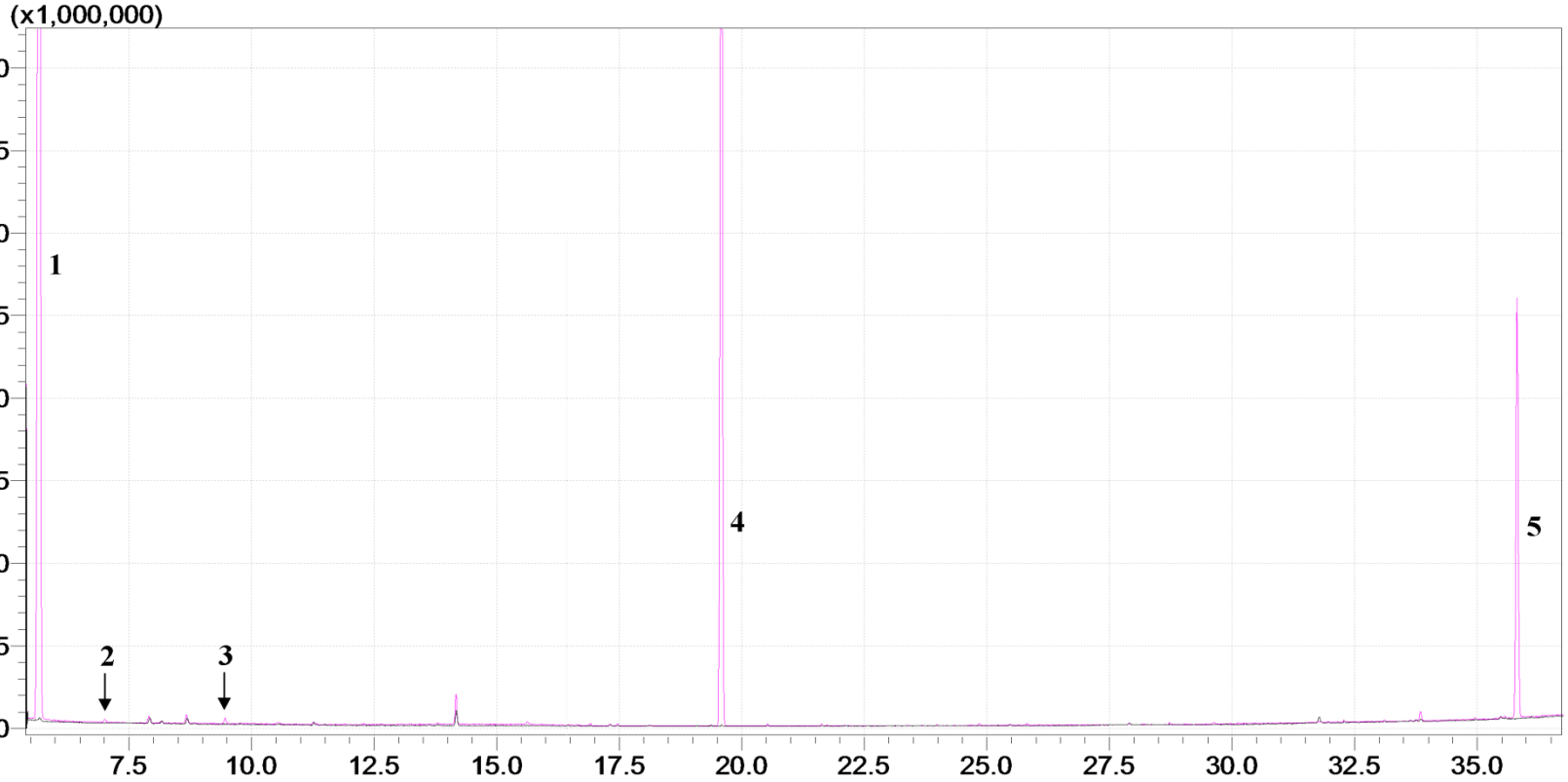
Appendix 3. Representative chromatogram for staminate flower zone of the species of Calyptrata complex (*S. baangongensis*) that analyzed by BP20 polar column in split mode. 1= 3-Butenoic acid, 3-methyl-, methyl ester; 2= Methyl benzoate.



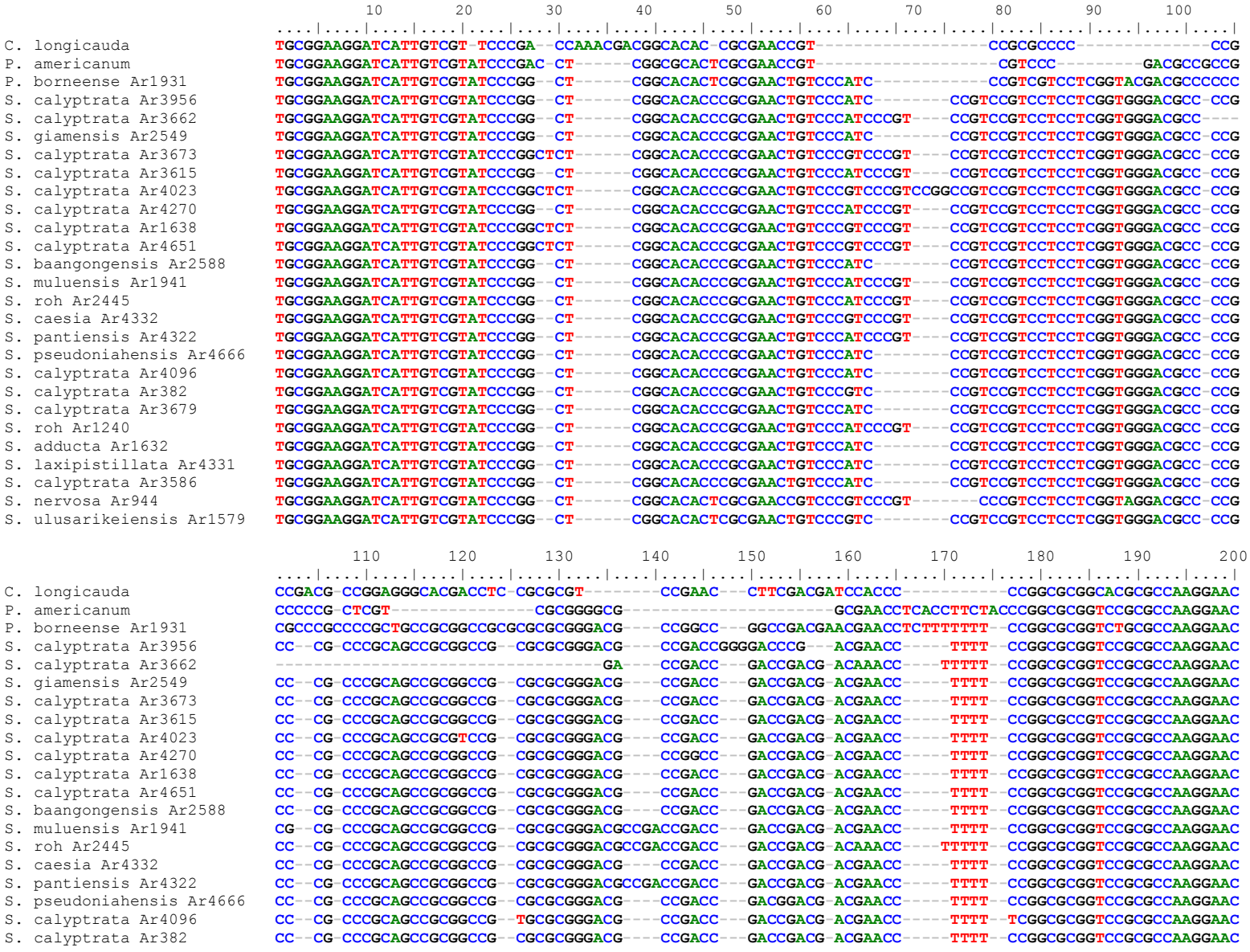
Appendix 4. Representative chromatogram for pistillate flower zone of the species of Calyptrata complex (*S. baangongensis*) that analyzed by BP20 polar column in split mode. 1= 3-Butenoic acid, 3-methyl-, methyl ester; 2= Methyl benzoate.

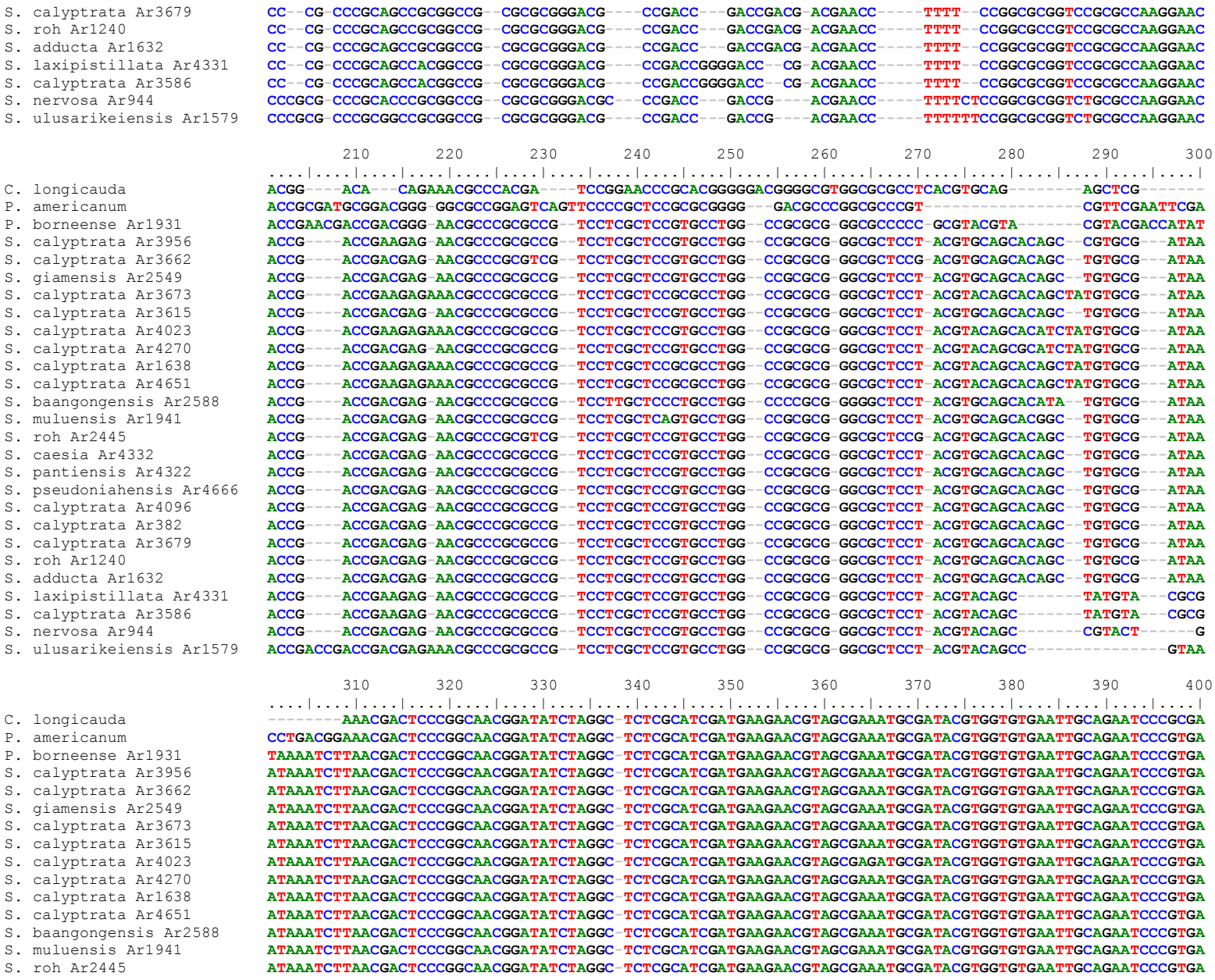


Appendix 5. Representative chromatogram for spathe of the species of Calyptrata complex (*S. baangongensis*) that analyzed by BP20 polar column in split mode. 1= 3-Butenoic acid, 3-methyl-, methyl ester; 2= 2-Butenoic acid, 3-methyl-, methyl ester; 3= 3-Buten-1-ol, 3-methyl-; 4= Benzoic acid, methyl ester; 5= Indole.



Appendix 6. Total Aligned ITS Region Sequences for the investigated species of Calyptrata complex.

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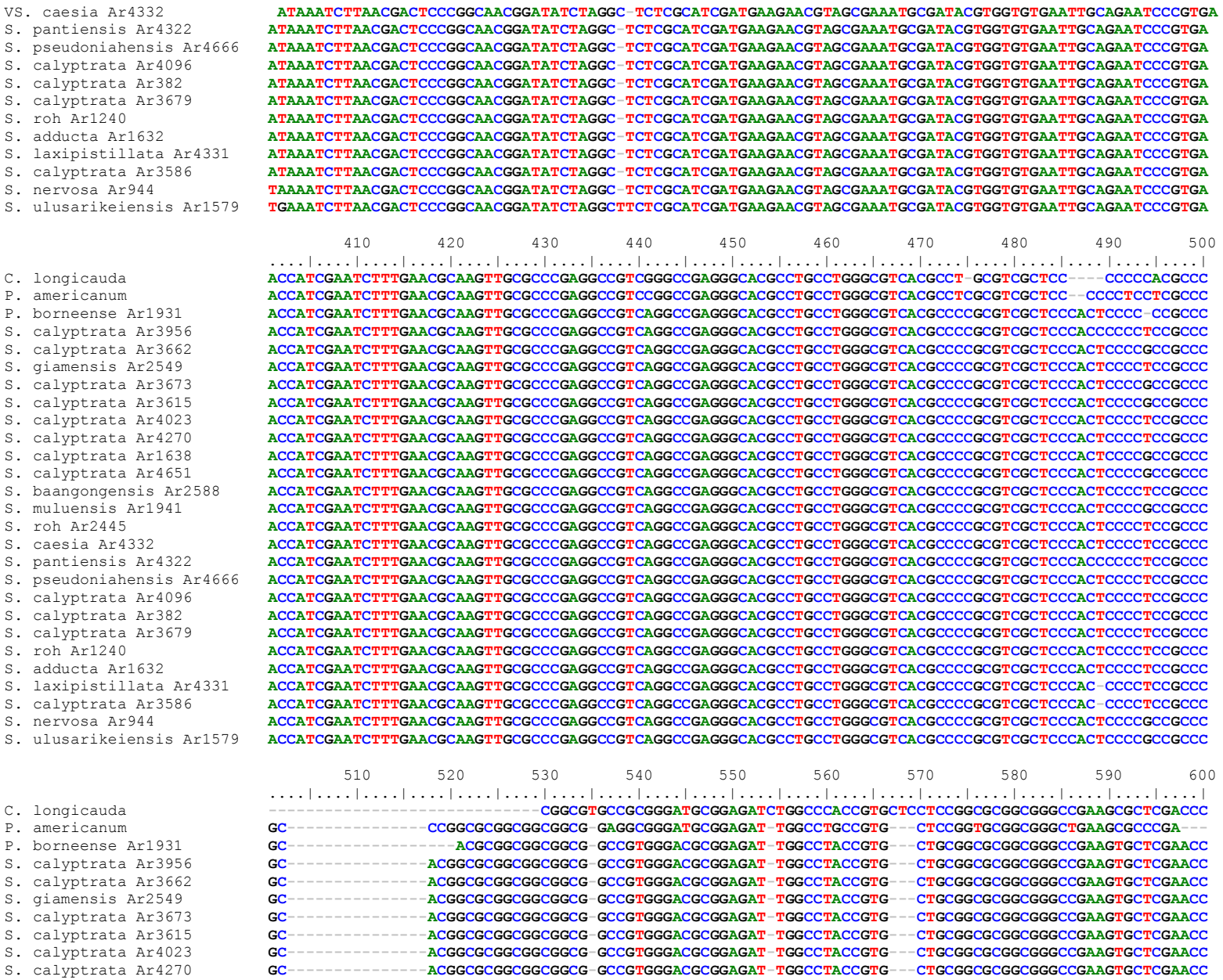
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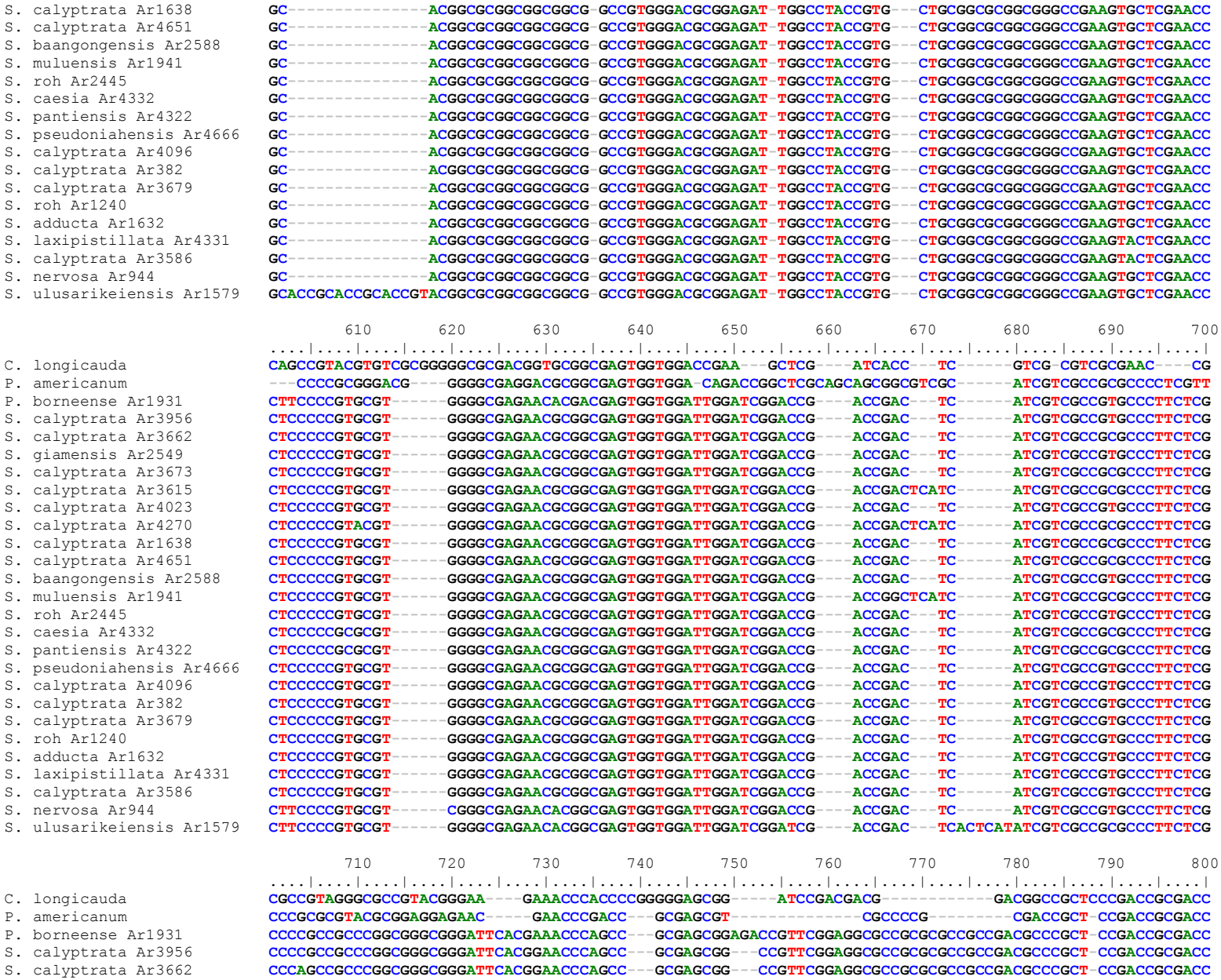
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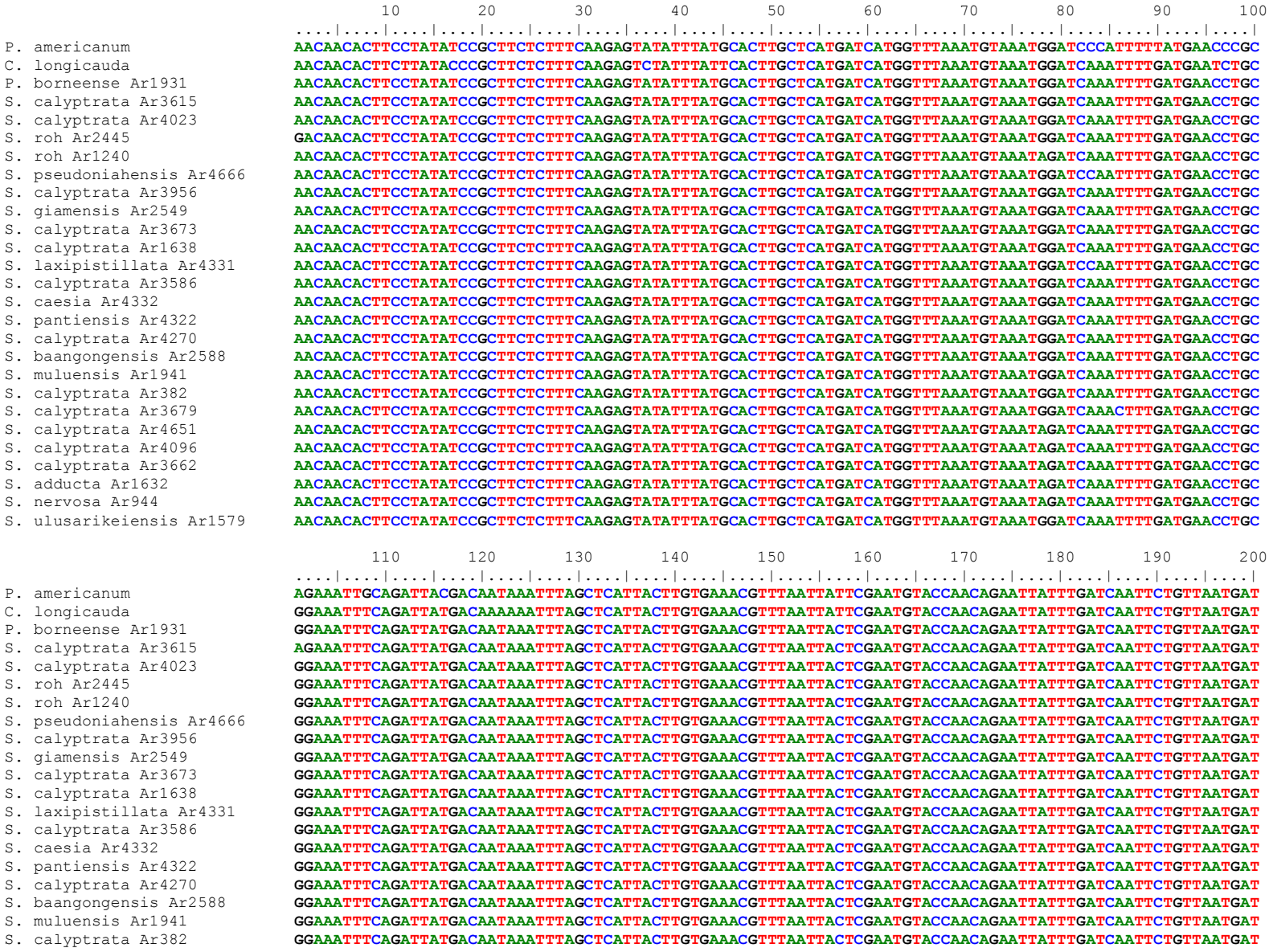

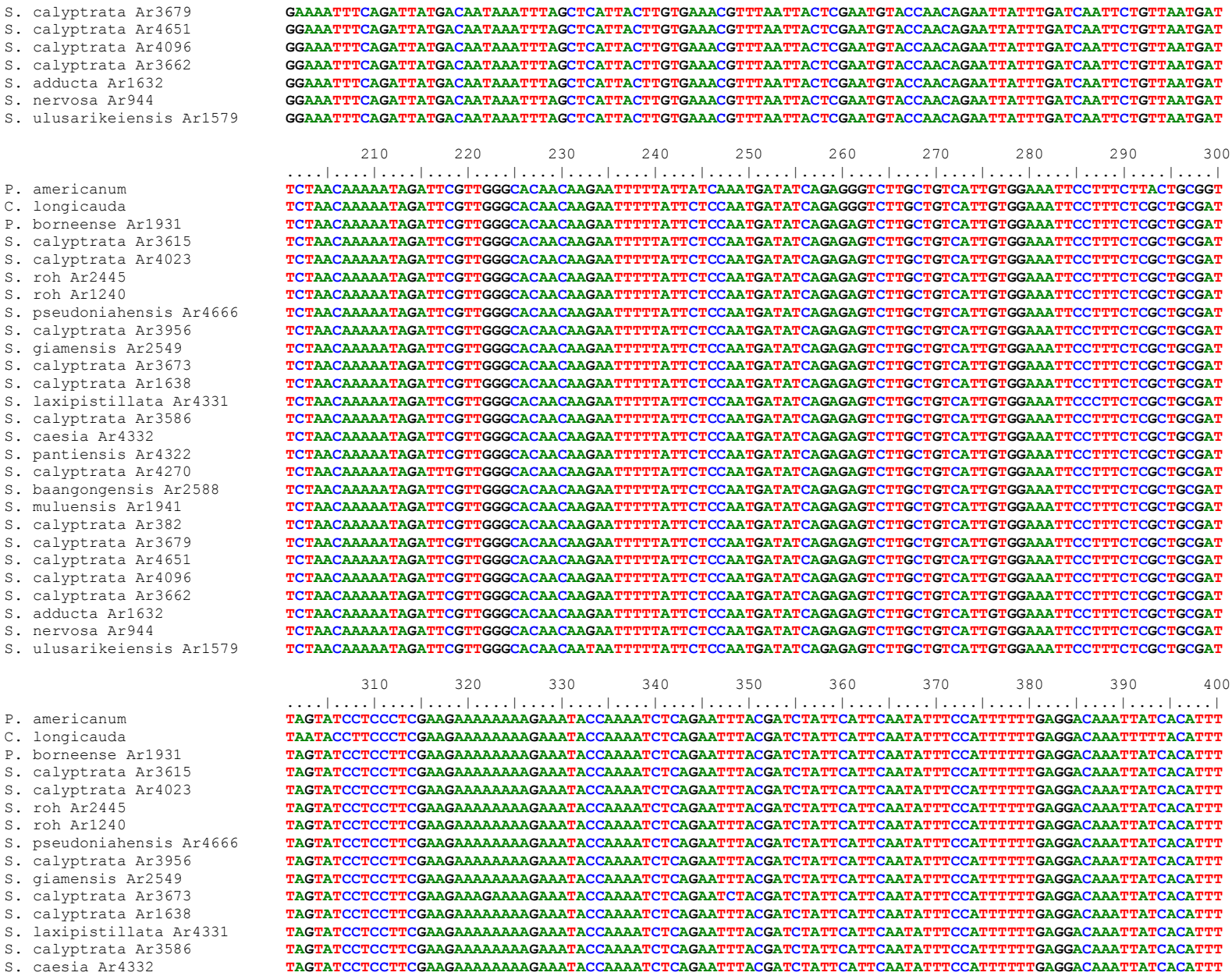
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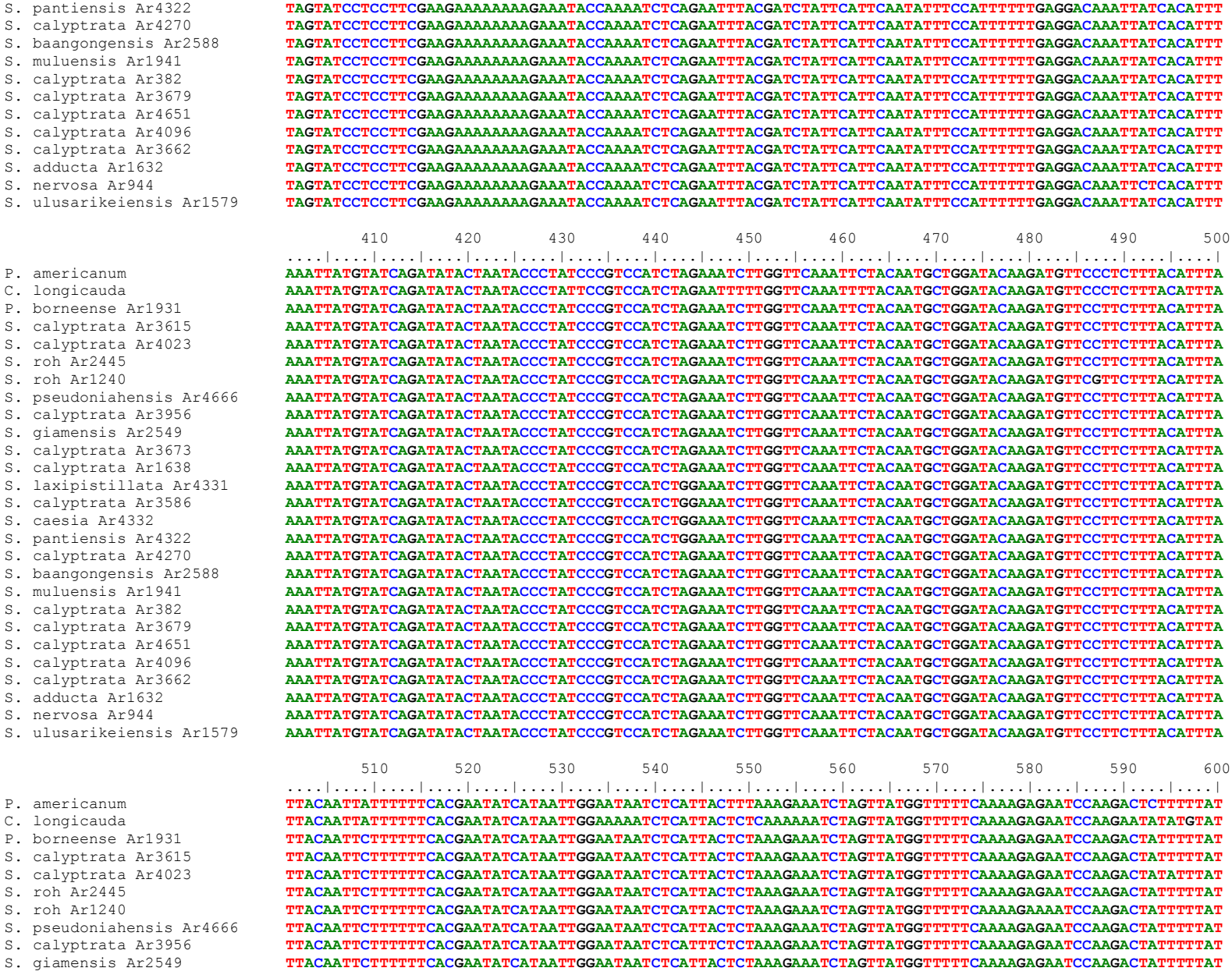
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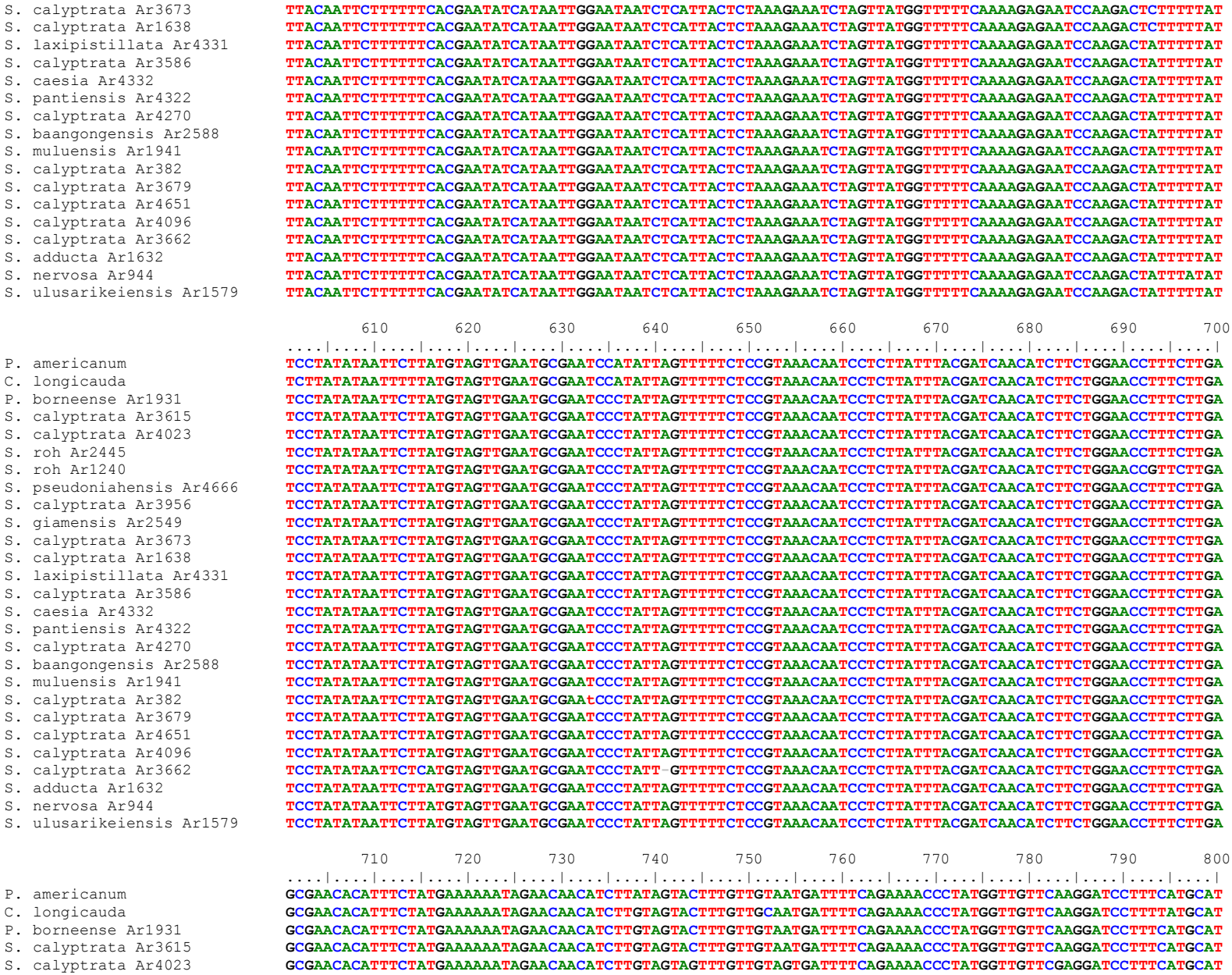
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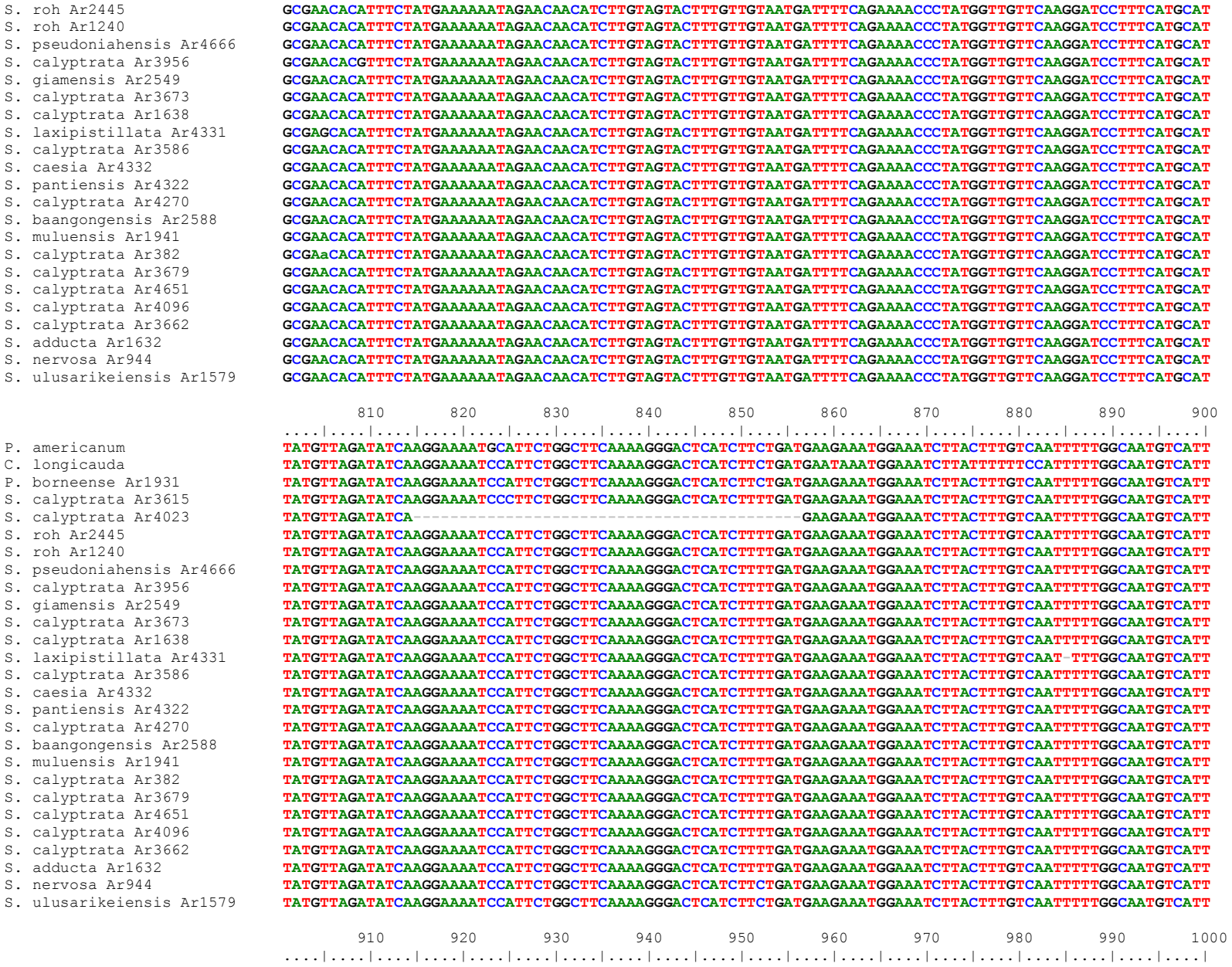
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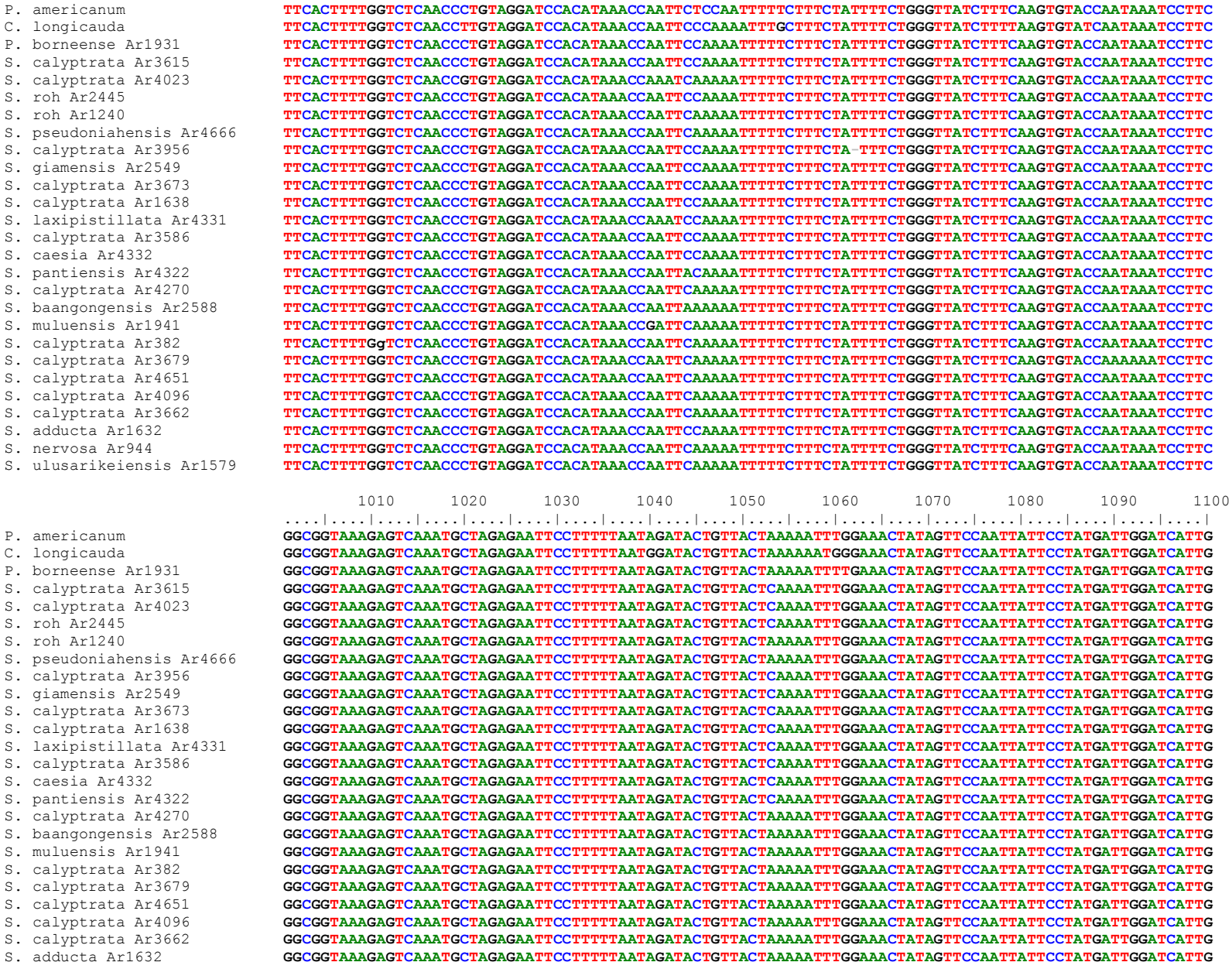
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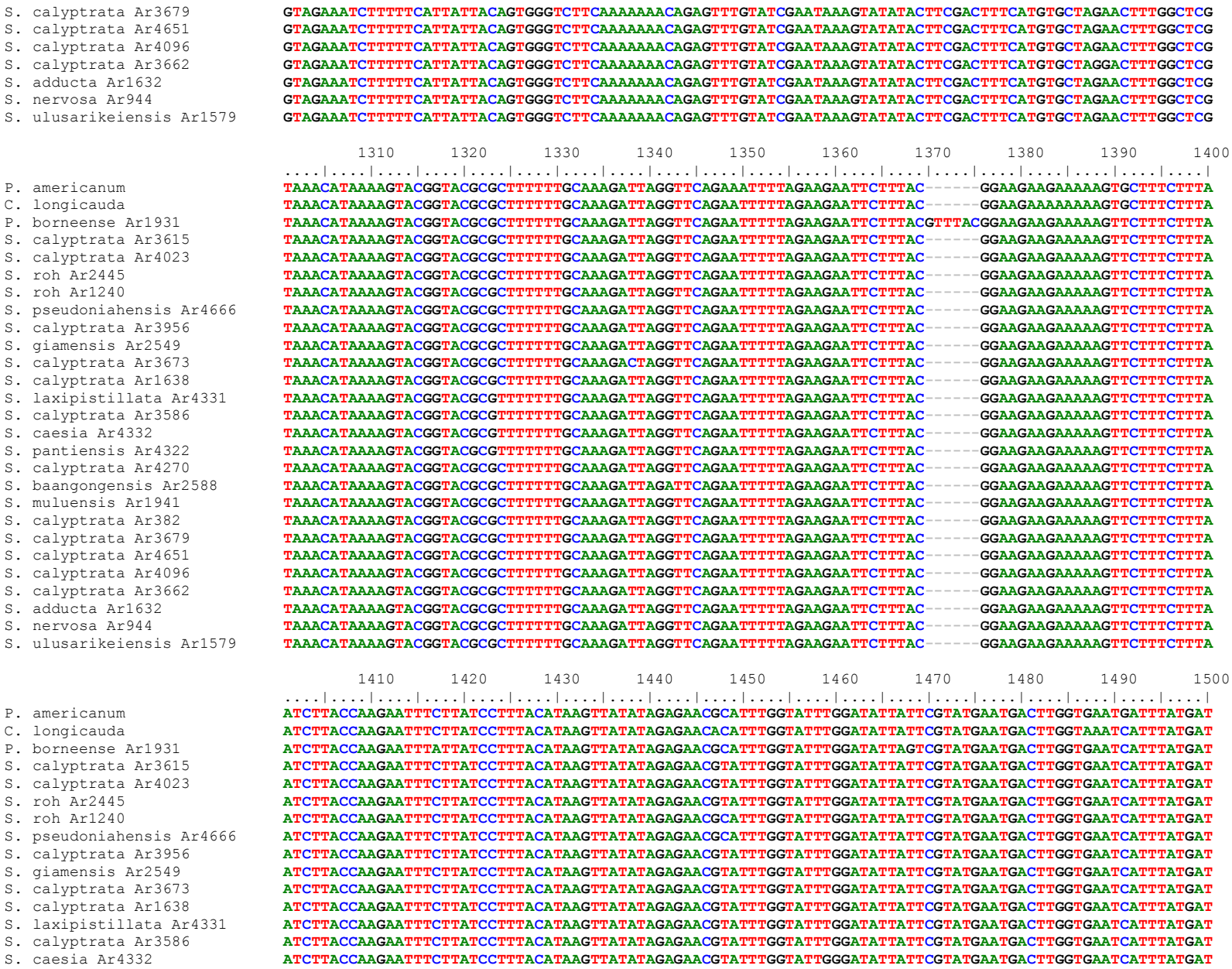
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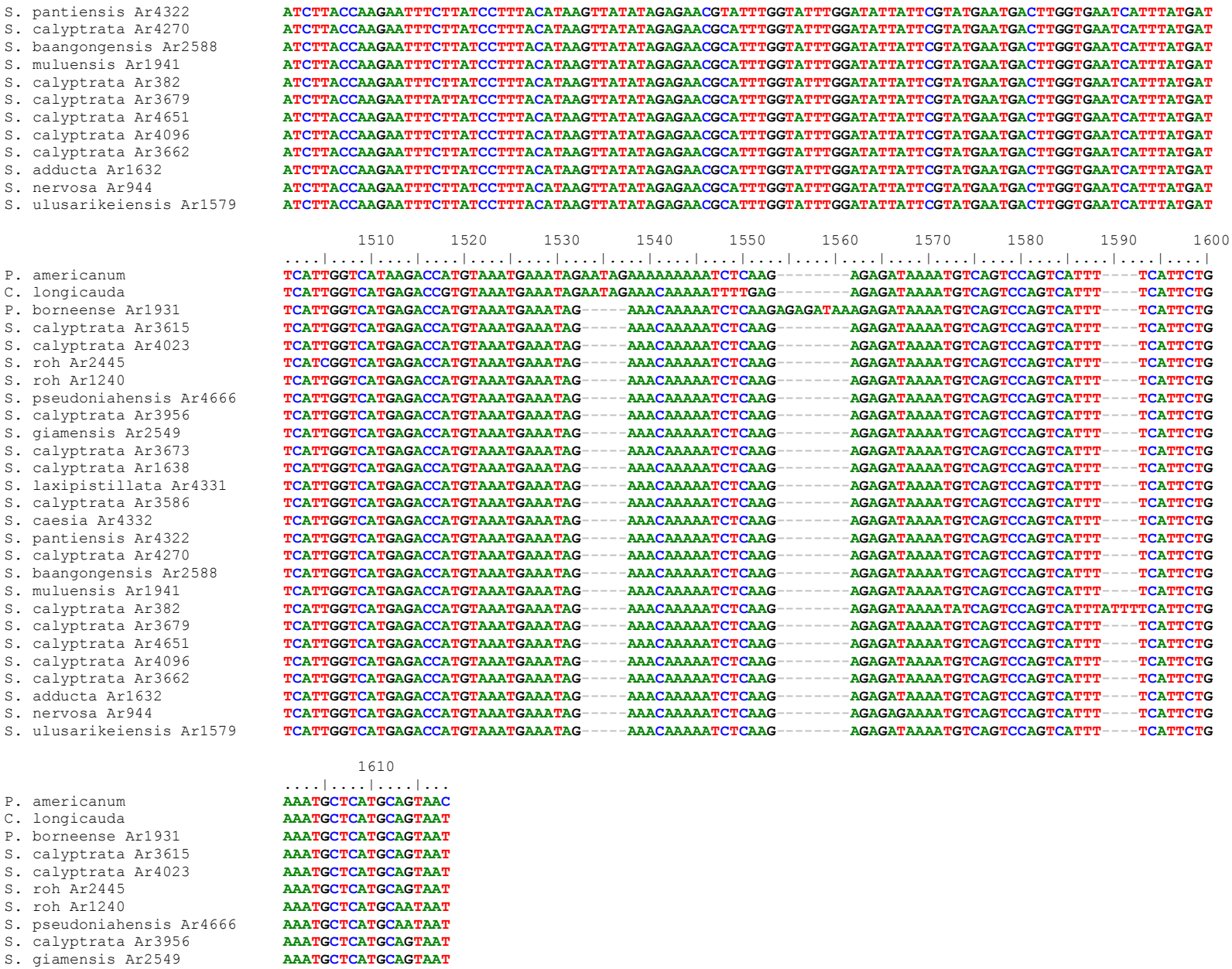
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